I. EVIDENCE APPENDIX

1. Autret *et al.* ("Autret") 1991, "Comparison of the plasmatic concentration and the tolerance of a single dose of human calcitonin following intradermal and subcutaneous administration," Therapie 46, 5-8 (with English language translation). This reference was admitted into the record by the examiner on 15 June 2004 (in the Office Action dated 06/15/04).

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12. Declaration of Dr. Ronald J. Pettis under 37 C.F.R. 1.132, 6 January 2005 ("First Pettis Decl."). This declaration was admitted into the record by the examiner on 7 April 2005 (in the Office Action dated 04/07/05).

13. Declaration of Dr. Ronald J. Pettis under 37 C.F.R. 1.132, 6 October 2005 ("Second Pettis Decl."). This declaration was admitted into the record by the examiner on 23 February 2006 (in the Office Action dated 02/23/06).

14. Declaration of Dr. Ronald J. Pettis under 37 C.F.R. 1.132, 15 June, 2007 ("Third Pettis Decl."). This declaration was admitted into the record by the examiner on 31 October 2007 (in the Office Action dated 10/31/07).

15. Amendment under 37 C.F.R. 1.111, dated 7 October 2005, filed in response to the office action mailed on 7 April 2005. This amendment was admitted into the record by the examiner on 23 February 2006 (in the Office Action dated 02/23/06).

16. Amendment under 37 C.F.R. 1.111, dated 18 June 2007, filed in response to the office action mailed on 4 January 2007. This amendment was admitted into the record by the examiner on 31 October 2007 (in the Office Action dated 10/31/07).

17. Copy of Application No: 09/606,909 as filed on 29 June 2000.

Comparaison des concentrations plasmatiques et de la tolérance d'une dose unique de calcitonine humaine administrée par voie intradermique et sous-cutanée

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Résumé. L'objectif de l'étude était de comparer les concentrations plasmatiques de calcitonine obtenues après l'administration intradermique (ID) et sous-cutanée (SC) de Cibacalcine®.

Six volontaires sains âgés de 18 à 40 ans ont reçu de la Cibacalcine® par voie ID et SC dans un ordre aléatoire déterminé par tirage au sort mais au même endroit d'injection. Chaque administration était séparée de 8 jours. Les taux plasmatiques de calcitonine ont été mesurés par R.I.A. avant administration (T0) puis 2, 7, 15, 30 minutes et 1, 2, 4, 8, 12 heures après chaque administration.

La concentration maximum (C_{max}) était observée à 23,6 ± 10 min et 16,2 ± 7,5 min respectivement pour la voie SC et la voie ID. Avec la voie ID, le pic plasmatique était obtenu en même temps ou plus tôt (3 fois). La C_{max} est significativement supérieure avec la voie ID qu'avec la voie SC. Ni la concentration plasmatique aux différents temps de mesure, ni les aires sous la courbe entre 0 et 1 440 min ou entre 0 et 480 min (cette dernière étant utilisée pour minimiser l'importance du taux physiologique de calcitonine) ne sont significativement différents avec la voie ID et SC. Seize effets secondaires modérés (8 avec chaque voie d'administration) ont été observés chez 4 sujets. Ils sont apparus dans les 5 premières minutes et ont été transitoires (1 heure) pour les manifestations cutanées ; et ils ont été plus retardés entre 3 et 7 heures pour les nausées, les vomissements et l'asthénie. L'abaissement maximum de la calcémie enregistrée a été de 0,29 ± 0,21 et 0,18 ± 0,11 mmol/l respectivement par la voie SC et ID.

Dans cette étude, les voies ID et SC ne donnent de résultats différents ni pour les taux plasmatiques, ni pour les effets secondaires.

Mots-clés : Calcitonine humaine ; Voie d'administration intradermique ; Effet secondaire ; Taux plasmatique.

Comparison of the plasmatic concentration and the tolerance of a single dose of human calcitonin following intradermal and subcutaneous administration. Intradermal calcitonin

Summary. *The aim of the study was to compare plasmatic levels of calcitonin obtained after intradermic (ID) and usual subcuteneous (SC) route of administration.*

Patients - Method : 6 volunteers between 18 and 40 years old received calcitonin 0.5 mg (Cibacalcin, Ciba-Geigy Laboratory) by ID and SC routes in a random order but at the same site of injection. Each administration was spaced by 8 days. Plasmatic levels were measured by R.I.A. before administration then 2 (M2), 7 (M7), 15 (M15), 30 (M30) minutes and 1 (H1), 2 (H2), 4 (H4), 8 (H8), 12 (H12) hours after each administration.

Results : T_{max} were at 23.6 ± 10 min and 16.2 ± 7.5 min for SC and ID routes respectively. With ID route, T_{max} was reached simultaneously or earlier (3 times). C_{max} is significantly higher with ID route. Neither mean plasmatic levels at each plasmatic dosage nor mean areas under the curve between 0 to 1 440 min or between 0 to 480 min (this latest AUC calculate to minimize the importance of calcitonin basal level) were significantly different with ID and SC routes. 16 mild side effects (8 with each administration) were observed in 4 subjects. They appeared before 5 min. and were transient (1 hour) for cutaneous manifestations, and later between hours 3 and 7 for nausea and vomiting or asthenia. The mean lowering of calcemia was 0.29 ± 0.21 and 0.18 ± 0.11 mmol/l respectively for SC and ID route.

In this study ID and SC routes for calcitonin administration are not different with regard to plasma levels and side effects.

Key-words : *Human calcitonin ; Intradermic administration ; Side effect ; Plasmatic level.*

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Therapie 1991 ; *46 :* 5-8

La Cibalcacine® (Ciba-Geigy) calcitonine humaine de synthèse intervient dans la régulation du métabolisme osseux en inhibant l'action des ostéoclastes. Elle est indiquée dans les maladies osseuses s'accompagnant d'hyper-résorption comme la maladie de Paget, l'algodystrophie. La voie d'administration la plus couramment utilisée est la voie sous-cutanée (SC) (1, 2). Plus récemment la voie intradermique (ID) par technique mésothérapique a été proposée sur des arguments non documentés d'une résorption plus rapide et supérieure avec cette voie d'administration. L'objectif de cette étude était de comparer les concentrations plasmatiques de calcitonine après l'administration de Cibacalcine® par voie ID (méthode de mésothérapie) et par voie SC.

MÉTHODOLOGIE

L'étude a été menée chez le volontaire sain : peuvalent être inclus les sujets de sexe masculin ou féminin, âgés de 18 à 40 ans, n'ayant pris aucun médicament dans le mois précédant l'étude et sans antécédent personnel ou familial d'allergie.

Chaque sujet était son propre témoin et recevait successivement et dans un ordre aléatoire attribué par tirage au sort, une ampoule de Cibacalcine® par voie ID et SC. Les deux séquences étaient séparées de 8 jours. L'essai a été effectué en ouvert mais la personne effectuant les dosages de calcitonine ignorait sa voie d'administration.

L'évaluation portait sur la concentration maximale (C_{max}), le temps pour obtenir la C_{max} (T_{max}), l'aire sous la courbe des concentrations plasmatiques en fonction du temps (AUC). Pour la comparaison des T_{max} et C_{max} un test non paramétrique pairé (Wilcoxon) a été affectué. La demi-vie (T 1/2) a été calculée entre la C_{max} et la 4e h.

Les sujets étaient hospitalisés pendant 12 h, un cathéter était posé au pli du coude gauche pour les prélèvements plasmatiques. La Cibacalcine® 0,5 mg diluée dans une ampoule de 1 ml de mannitol à 3 % était injectée : soit en intradermique à l'aide d'une aiguille de Lebel à usage unique de 4 mm/0,4 mm : 5 injections simultanées de 0,2 ml chacune au niveau de la région deltoïdienne droite, l'aiguille étant enfoncée jusqu'à la garde selon un angle d'environ 60° ; soit en SC à l'aide d'une aiguille à usage unique de 16 mm/0,5 mm : une injection de 1 ml dans la région deltoïdienne droite en piquant parallèlement la peau dans un pli cutané entre 2 doigts. Toutes les injections ont été faites par le même investigateur, pratiquant couramment la mésothérapie.

Deux ml de sang ont été prélevés pour dosages plasmatiques de la calcitonine et de la calcémie aux temps suivants : 15 min avant injection de Cibacalcine® (T0), puis 2, 7, 15, 30, 60 minutes et 2 (H2), 4 (H4), 8 (H8), 12 (H12) heures après la fin de l'injection. Le cathéter étant ensuite enlevé et les sujets revenaient le lendemain matin pour le dernier prélèvement de 24 h.

La calcitonine a été dosée par méthode radioimmunologique (3) ; l'anticorps utilisé obtenu chez le mouton immunisé par la calcitonine humaine de synthèse (Ciba-Geigy) est dirigé essentiellement contre la partie médiane de la molécule. Le traceur est la calcitonine radiomarquée à l'iode 125. Le plasma ou les solutions étalons étaient incubés en présence de l'anticorps pendant 24 h à 4 °C puis de nouveau 24 h en présence du traceur. A la fin de ces incubations la calcitonine liée à l'anticorps était séparée de la calcitonine libre par une technique au charbon-dextran. Le signal B/T (radioactivité forme liée/radioactivité totale) a permis de déterminer la concentration de l'échantillon en se reportant à une courbe d'étalonnage réalisée dans les mêmes conditions ; la limite de détection de cette technique est de l'ordre de 20 pg/ml. Les variations de la calcémie ont été analysées chez chaque sujet par le rapport AUC_{0-8h} observé/AUC_{0-8h} théorique en l'absence de modification de la calcémie de base calculée par le produit calcémie de base par 8 h. La valeur de la calcémie de base est la moyenne des calcémies H0 et H24.

Les manifestations cliniques anormales ont été recueillies de façon spontanée. Le consentement écrit des sujets était demandé et le protocole a reçu l'aval du Comité d'Ethique du CHU de Tours.

RÉSULTATS

Six volontaires sains (4 hommes et 2 femmes), de poids de 69 ± 11,2 kg (49-80) et de taille 1,73 ± 0,1 m (1,53-1,80) ont participé à l'étude.

Les concentrations plasmatiques moyennes de calcitonine *(fig. 1)* avant toute administration à T0 (135 ± 67 vs 154 ± 79 pg/ml) sont comparables dans les 2 groupes. La moyenne ± 1 DS des T1/2 est à 0,71 ± 0,16 h et 0,87 ± 0,4 h avec la voie SC et ID respectivement.

Le T_{max} est à 23,6 ± 10 min avec la voie SC et 16,2 ± 7,5 min avec la voie ID. Le test T de Wilcoxon n'a pu être réalisé car pour 2 paires la différence est

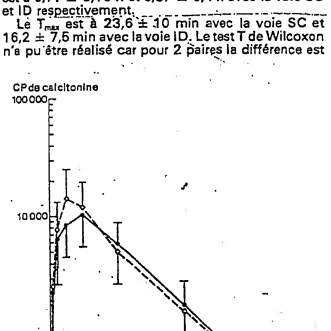

FIG. 1. — *Concentrations plasmatiques (CP) de calcitonine par voie intra-dermique (O···O) et sous-cutanée (●——●).*

TABLEAU I. — *Aire sous la courbe (AUC) (en ng/h/ml) des concentrations plasmatiques de calcitonine en fonction du temps avec la voie intradermique (ID) et sous-cutanée (SC).*

Sujet n°	AUC_{0-24h}			AUC_{0-4h}			AUC_{0-4h}		
	ID	SC	BD	ID	SC	BD	ID	SC	BD
1	15,50	15,46	(1)	3,70	14,13	(0,96)	13,44	13,78	(0,97)
2	6,62	9,39	(0,7)	4,10	7,73	(0,53)	3,27	6,87	(0,47)
3	13,69	11,91	(1,15)	11,86	9,97	(1,19)	11,18	9,06	(1,23)
4	7,05	7,58	(0,93)	6,24	6,82	(0,91)	5,87	6,17	(0,95)
5	9,49	9,26	(1,02)	8,36	7,54	(1,11)	8,02	8,43	(0,95)
6	13,87	8,32	(1,66)	12,33	7,17	(1,72)	11,76	6,86	(1,71)
	NS			NS			NS		
m	11,04	10,32	(1,07)	9,43	8,89	(1,07)	8,92	8,53	(1,04)
ET	3,81	2,91	(0,32)	3,80	2,79	(0,39)	3,89	2,79	(0,41)

m : moyenne ; ET : écart-type ; BD : biodisponibilité relative de la voie ID par rapport à la voie SC ; NS : non significatif.

nulle et les 4 paires restantes représentent un effectif insuffisant. Le T_{max} est plus précoce pour 3 sujets (n°s 1, 3 et 4) avec la voie ID à 30, 15, 15 min respectivement tandis que pour 3 autres elle apparaît en même temps à 7, 30, 15 min quelle que soit la voie d'administration. La C_{max} moyenne est significativement plus élevée ($p < 0,05$) avec la voie ID (m ± 1 DS = 16 660 ± 8 545 pg/ml) qu'avec la voie SC (11 666 ± 5 456 pg/ml). Les concentrations plasmatiques aux différents temps ne sont pas significativement différents avec les voies d'administration *(fig. 1)*. A partir de la 4e h, les concentrations plasmatiques de calcitonine redeviennent proches des concentrations de base : la calcitonine endogène est donc sûrement en partie en cause. En effet la Cibacalcine® étant strictement analogue au monomère de calcitonine sécrété par les cellules de la thyroïde, la méthode de dosage ne permet pas de distinguer calcitonine endogène et exogène. Pour minimiser ce phénomène, les analyses des AUC ont été faites entre T0 et H4 et T0 et H8 en plus des AUC de T0 à 24 h. Les AUC obtenues avec les 2 voies d'administration calculées entre T0 et successivement .H24, H8, H4 ne sont pas différentes *(tableau I)*.

Seize manifestations cliniques ont été observées chez 4 sujets, 8 avec la voie SC et 8 avec la voie ID. Leur type et leur durée apparaissent dans le *tableau II* ; elles ont été modestes et il s'agissait essentiellement de troubles digestifs (3), de paresthésies (5) et de « flush » (5). Elles ont été immédiates et de brève durée, inférieures à 1 h pour les fourmillements, le « flush » et la réaction locale ; plus retardées (après la 3e h) et de durée plus prolongée (2 à 3 h) pour les nausées, les vomissements et l'asthénie.

Deux sujets (n°s 3 et 5) n'ont eu aucune manifestation anormale quelle que soit la voie d'administration ; les 4 autres ont eu les mêmes manifestations locales avec les 2 voies d'administration ; de plus le sujet n° 4 a eu une réaction locale à la suite de la voie ID tandis que le patient n° 2 a eu des troubles digestifs au décours de l'injection SC. La pression artérielle et la fréquence cardiaque sont restées stables pendant toute la durée de l'étude.

TABLEAU II. — *Manifestations cliniques observées avec la voie d'administration sous-cutanée (SC) et intradermique (ID).*

	SC		ID	
	Effectif	Durée des signes	Effectif	Durée des signes
Fourmillement au niveau du visage et des extrémités	3	2-15 min	2	2-30 min
« Flush » du visage et des oreilles	2	2-30 min	3	7-60 min
Asthénie	1	5-7 h	1	5-7 h
Nausée-vomissements	1	3-4 h	2	4-7 h
Réaction locale	1	2-60 min	0	—

Deux sujets (n°s 3 et 4) avaient des calcémies basses (< 2,2 mmol/l) avant l'administration de calcitonine. Les variations de la calcémie par rapport à la calcémie de T0 *(tableau III)* ne sont disponibles que 5 fois avec la voie SC et 4 fois avec la voie ID en raison de l'absence de mesure à T0 chez 3 sujets.

TABLEAU III. — *Variation des calcémies en fonction du temps et selon la voie d'administration.*

Sujet n°	T0	7 min	30 min	H2	H8	H12	H24	Abais. max. (mmol/l)
Voie sous-cutanée								
1	2.42	—	2.38	2.17	2.24	—	2.3	0.25
2	2.43	2.25	2.39	2.22	2.24	2.35	2.5	0.21
3	2.18	2.21	2.18	2.13	2.23	2.16	1.94	0.24
4	2.04	1.94	1.86	1.80	2.02	2.06	—	0.24
5	2.37	2.32	2.39	—	2.34	2.35	2.33	0.09
6			1.7	2.07	2.13	—	—	—
Voie intradermique								
1	2.42	2.3	2.38	2.32	2.36	—	2.48	0.12
2	2.45	2.22	2.32	2.23		—	2.22	0.23
3	—	2.22	2.19			2.25	2.2	—
4	2.07	—	2.16	2.2	2.04	2.05	2.2	0.03
5	—	—	2.02	—	—	—	2.17	—
6	2.39	2.44	2.38	2.15	2.26	2.49	2.38	0.24

Avec la voie SC les diminutions les plus importantes sont 3 fois enregistrées à la 2e h et 1 fois à H1 et H24 ; elles sont comprises entre 0,09 mmol et 0,25 mmol/l (m ± 1 DS = 0,20 ± 0,06). Avec la voie ID cet abaissement de la calcémie est maximum 2 fois à 7 min et 1 fois à H2 et H8 ; il est compris entre 0,03 mmol/l et 0,24 mmol/l (m ± 1 DS = 0,15 ± 0,09). Le rapport $AUC_{0\text{-}8h}$ observé/$AUC_{0\text{-}8h}$ théorique de le calcémie n'est pas différent avec la voie ID (0,98 ± 0,02) et SC (0,98 ± 0,02).

DISCUSSION

Il existe une importante variation interindividuelle des concentrations plasmatiques de calcitonine comme en témoigne l'écart-type important des moyennes des concentrations aux différents temps chez les 6 volontaires. En revanche, il n'y a pas de différence chez un même sujet entre les 2 voies d'administration en ce qui concerne les T_{max}, AUC et variations de la calcémie. Seule la C_{max} est supérieure avec la voie ID. Il n'y a pas d'études contrôlées permettant d'affirmer l'efficacité avancée par les praticiens en mésothérapie. Ces données vont à l'encontre d'une résorption plus rapide ou supérieure mise en avant pour justifier la mésothérapie. Cependant les mésothérapeutes n'utilisent que rarement la Cibacalcine® seule mais l'associent volontiers à d'autres substances telles que la procaïne.

La littérature est pauvre en données pharmacocinétiques concernant la calcitonine (4, 5). Dans l'étude de Nuesch et Schmidt (6) la C_{max} survient une 1/2 h après une injection SC. A notre connaissance, notra étude est la première après administration ID.

Les effets indésirables observés chez 66 % des patients sont similaires quelle que soit la technique d'injection. Ils sont précoces, de brève durée et bénins comme ceux rapportés par Mc Intyre *et al.* (1). Ainsi, ni la pharmacocinétique, ni les effets secondaires de la calcitonine ne différencient la voie ID de la voie SC.

En revanche, on peut s'interroger sur l'opportunité de multiplier le nombre d'effraction cutanée, et donc les risques infectieux (7), pour un résultat non différent d'une administration unique.

CONCLUSION

La mésothérapie n'a jamais été l'objet d'études cliniques contrôlées. Une meilleure résorption de la Cibacalcine® avancée par certains auteurs comme preuve indirecte n'a pu être mise en évidence. Cette étude montre que la calcitonine humaine à une C_{max} supérieure mais une biodisponibilité non différente après injection ID (mésothérapie) qu'après l'administration SC classique. Les effets secondaires, bénins, sont de fréquence égale dans les 2 cas.

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Synthetic human calcitonin Cibacecine® [sic – probably Cibacalcine®] (Ciba-Geigy) intervenes in the regulation of the osseous metabolism, inhibiting the action of osteoclasts. It is indicated in osseous diseases accompanied by hyper-resorption such as Paget's disease and algodystrophy. The most currently used route of administration is the subcutaneous route (SC) (1,2). More recently, the intradermic route (ID) by mesotherapic technique has been proposed based on non-documented arguments of a more rapid and superior resorption with this route of administration. The object of this study was to compare the plasmatic concentrations of calcitonin after the administration of Cibacalcine® by [via] the ID route (mesotherapy method) and by the SC route.

METHODOLOGY

The study was carried out on healthy volunteers. Male or female subjects from 18 to 40 years of age were able to be included who had not taken any medicament in the month preceding the study and without personal or familial antecedent.

Each subject was his own check and received successively and in a random order established by drawing lots one ampoule of Cibacalcine® by the ID and the SC route. The two sequences were separated by 8 days. The

trial was carried out openly but the person making the dosages of calcitonin did not know its route of administration.

The evaluation concerned the maximum concentration (C_{max}), the time to obtain the C_{max} (T_{max}), the area under the curve of plasmatic concentrations as a function of the time (AUC). A non-parametric, paired test (Wilcoxon) was made to compare the T_{max} and the C_{max}. The half-life ($T_{½}$) was calculated to be between the C_{max} and the 4th h.

The subjects were hospitalized for 12 h, a catheter was placed at the fold of the left neck for plasmatic samples. 0.5 mg Cibacalcine® diluted in an ampoule of 1 ml mannitol at 3 % was injected: either intradermically with the aid of a Lebel single-usage needle with 4 mm/0.4 mm: 5 simultaneous injections of 0.2 ml each at the level of the right deltoidal region with the needle being inserted up to the guard at an angle of approximately 60°; or by SC with the aid of a single-usage needle with 16 mm/0.5 mm : one injection of 1 ml in the right deltoidal region by a parallel piercing of the skin in a cutaneous fold between 2 fingers. All the injections were made by the same researcher currently practicing mesotherapy.

Two ml of blood were taken for plasmatic dosages of the calcitonin and of the calcemia at the following times: 15 min prior to the injection of Cibacalcine® (TO), then 2,k 7, 15, 30, 60 minutes and 2 (H2), 4 (H4), 8 (H8) and 12 (H12) hours after the end of the injection. The catheter was then removed and the subjects returned the following morning for the last sampling of 24 h.

The calcitonin was dosed by the radioimmunological method (3); the antibody used and obtained from a sheep immunized by synthetic human calcitonin (Ciba-Geigy) is directed essentially against the median part of the molecule. The tracer is calcitonin radioactively marked with iodine 125. The plasma or the standard [calibrating] solutions were incubated in the presence of the antibody for 24 h at 4 °C, then again for 24 h in the presence of the tracer. At the end of these incubations the calcitonin bound to the antibody was separated from the free calcitonin by a carbon-dextran technique. The B/T signal (bound form radioactivity / total radioactivity) permitted the determination of the concentration of the sample by referring to a calibration curve realized under the same conditions; the detection limit of this technique is on the order of 20 pg/ml. The variations of the calcemia were analyzed for each subject by the ratio of observed $AUC_{0\text{-}8h}$ / theoretical $AUC_{0\text{-}8h}$ in the absence of modification of the base calcemia calculated by the product base calcemia by 8 h. The value of the base calcemia is the average of the calcemias H0 and H 24.

The abnormal clinical manifestations were collected in a spontaneous manner. The written consent of the subjects was requested and the protocol received the backing of the Ethical Committee of the CHU of Tours.

RESULTS

Six healthy volunteers (4 men and 2 women) weighing 69 ± 11.2 kg (49 – 80) and 1.73 ± 0.1 m in height (1.53 – 1.80 participated in the study.

The average plasmatic concentrations of calcitonin (figure 1) before any administration at TO (135 ± 67 vs 154 ± 79 pg/ml) are comparable in the two groups. The average ± 1 DS of the T1/2 is at 0.71 ± 0.16 h and 0.87 ± 0.4 h with the SC and the ID route respectively.

The T_{max} is at 23.6 ± 10 min with the SC route and 16.2 ± 7.5 min with the ID route. The Wilcoxon T test was not able to be realized because for 2 pairs the difference is

CP of calcitonin

[See page 1.]

Time (h)

Figure 1 – plasmatic concentrations (CP) of calcitonin via intradermic route (0....0) and via subcutaneous route (• - •). [Please let me know if these bullets print out as they should on your current computers!]

TABLE I – Area under the curve (AUC) (in ng/h/ml) of the plasmatic concentrations of calcitonin as a function of time with the intradermic route (ID) and the subcutaneous route (SC).

Subject No.	
1	
2	[See page 2 for numeric data.]
3	
4	
5	
6	
m	
ET	

m: average; ET: deviation type; BD: relative bioavailability of the ID route relative to the SC route/ NS: not significant

zero and the remaining 4 pairs represent an insufficient absolute frequency [number]. The T_{max} is earlier for 3 subjects (No. 1, 3 and 4) with the ID route at 30, 15, 15 min respectively whereas for 3 others it appears at the same time at 7, 30, 15 min whatever the route of administration. The average C_{max} is significantly higher ($p < 0.05$) with the ID route ($m \pm 1$ DS = 16,660 $\pm$ 8,545 pg/ml) than with the SC route (11,666 $\pm$ 5,456 pg/ml). The plasmatic concentrations at the different times are not significantly different with the routes of administration (fig. 1). After the 4th h, the plasmatic concentrations of calcitonin become close to the base concentrations again: The endogenous calcitonin is therefore surely partly involved. As a matter of fact, as Cibacalcine® is strictly analogous to the monomer of calcitonin secreted by the cells of the thyroid, the dosage method does not permit a distinction to be made between endogenous calcitonin and exogenous calcitonin. In order to minimize this phenomenon, the analyses of the AUC were made between TO and H4 and TO and H8 over and above the AUC of TO at 24 h. The AUC obtained with the 2 routes of administration calculated between TO and successively H24, H8, H4 are not different (*table I*).

Sixteen clinical manifestations were observed in 4 subjects, 8 with the SC route and 8 with the ID route. Their type and duration appear in *table II*; they were modest and it was essentially a matter of digestive troubles (3), paresthesias (5) and of flush (5). They were immediate and of short duration, below 1 h for the tingling, the flush and the local reaction; more

delayed (after the 3d h) and of a more prolonged duration (2 to 3 h) for the nausea, vomiting and asthenia.

Two subjects (No. 3 and 5) did not have any abnormal manifestation, whatever the route of administration; the 4 others had the same local manifestations with the 2 routes of administration; moreover, subject No. 4 had a local reaction following the ID route whereas patient No. 2 had digestive problems during the decline [abatement] of the SC injection. The arterial pressure and the heart rate remained stable during the entire time of the study.

TABLE II. – Clinical manifestations observed with the subcutaneous route of administration (SC) and the intradermic route of administration (ID).

	SC		ID	
	Absolute frequency	Duration of signs	Absolute frequency	Duration of signs
Tingling at the level of the face and of the extremities	[See page 2 for numeric data.]			
Flush of the face and ears				

Asthenia
Nausea-vomiting
Local reaction

Two subjects (No. 3 and 4) had low calcemias (< 2.2 mmol/l) before the administration of calcitonin. The variations of the calcemia relative to the calcemia of TO (*table III*) are only available 5 times with the SC route and 4 times with the ID route due to the lack of measuring at TO in 3 subjects.

TABLE III. – Variation of the calcemias as a function of the time and of the route of administration

---

Subject No.	TO	7 min	30 min	H2	H8	H12	H24	Max. lowering (mmol/l)
Subcutaneous route								

1

2 [see page 2 for numeric data.]

3

4

5

6

Intradermic route

1

2

3

4

5

6

With the SC route the most significant diminutions are registered 3 times at the 2nd h and once at H1 and H24; they are comprised between 0.09 mmol and 0.25 mmol/l ($m \pm 1$ DS = 0.20 ± 0.06). With the ID route this lowering of the calcemia is maximum 2 times at 7 min and one time at H2 and H8; it is comprised between 0.03 mmol/l and 0.24 mmol/l ($m \pm 1$ DS = 0.15 ± 0.09). The ratio $AUC_{0\text{-}8h}$ observed/$AUC_{0\text{-}8h}$ theoretical of the calcemia is not different with the ID route (0.98 ± 0.02) and the SC route (0.98 ± 0.02).

DISCUSSION

There is a significant interindividual [personal] variation of the plasmatic concentrations of calcitonin, as the significant deviation type of the averages of the concentrations at the different times in the 6 volunteers testifies. On the other hand, there is no difference in one and the same subject between the two routes of administration as concerns the T_{max}, AUC and variations of the calcemia. Only the C_{max} is greater with the ID route. There are no controlled studies that permit the efficacy advanced by the practitioners of mesotherapy to be confirmed. These facts speak against a more rapid or greater resorption put forward to justify mesotherapy.

US006056716A

United States Patent [19]

D'Antonio et al.

[11] Patent Number: 6,056,716

[45] Date of Patent: May 2, 2000

[54] **HYPODERMIC FLUID DISPENSER**

[75] Inventors: **Nicholas F. D'Antonio**, Liverpool; **Linda F. D'Antonio**, Syracuse, both of N.Y.; **John T. Wagner**, Drexel Hill, Pa.

[73] Assignee: **D'Antonio Consultants International Inc.**, N.Y.

[21] Appl. No.: **08/738,303**

[22] Filed: **Oct. 25, 1996**

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/253,416, Jun. 3, 1994, Pat. No. 5,569,190, which is a continuation-in-part of application No. 07/818,235, Jan. 8, 1992, Pat. No. 5,318,522, which is a continuation-in-part of application No. 07/336,636, Apr. 7, 1989, Pat. No. 5,080,648, which is a continuation of application No. 07/059,620, Jun. 8, 1987, abandoned.

[51] **Int. Cl.**[7] **A61M 5/30**

[52] **U.S. Cl.** **604/68**; 604/134

[58] **Field of Search** 604/68, 72, 198, 604/207, 46, 47, 131, 134

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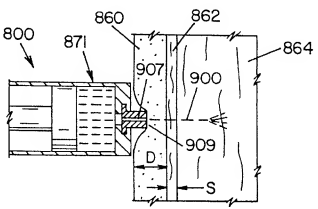
Primary Examiner—Ralph A. Lewis
Attorney, Agent, or Firm—D. Peter Hochberg; William H. Holt

[57] **ABSTRACT**

A jet injector system for injecting fluid into a body. The jet injection system includes capsules for holding the material to be injected, apparatus for applying force to the capsule(s) to eject the injection material(s) and a perforator for directing the jet stream for the respective materials into the body. A flyweight system is described for developing jet injection pressures, and latching devices control the flyweight system. An injector system for injecting more than one fluid is described.

18 Claims, 23 Drawing Sheets



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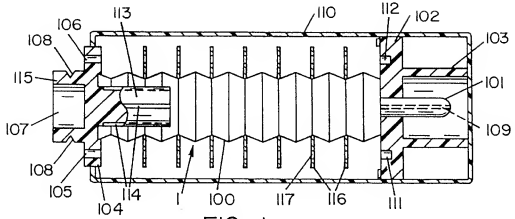

FIG. 1

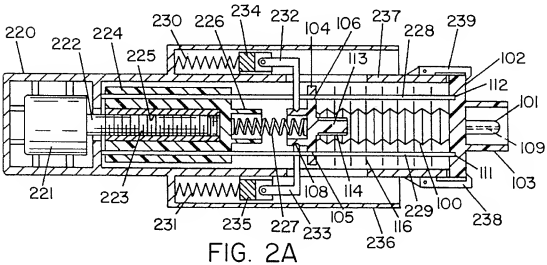

FIG. 2A

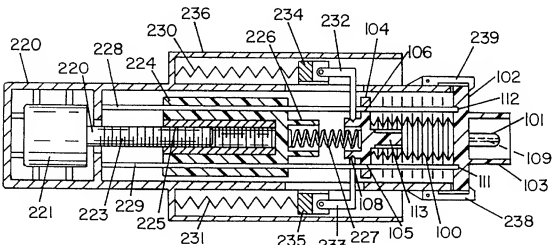

FIG. 2B

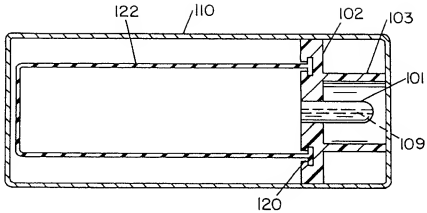

FIG. 1A

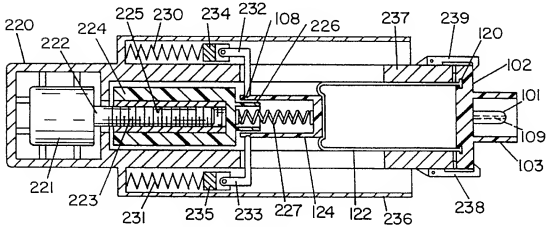

FIG. 2AA

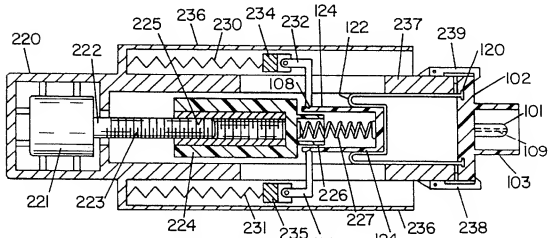

FIG. 2BB

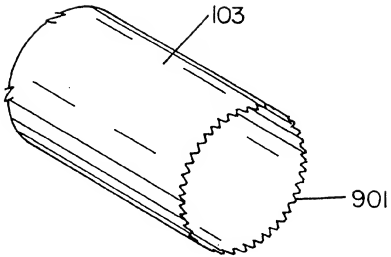

FIG. 1B

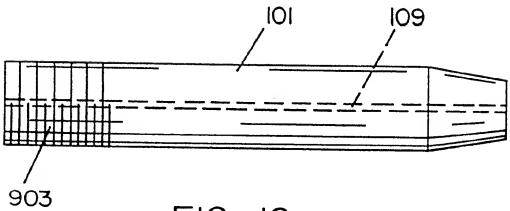

FIG. 1C

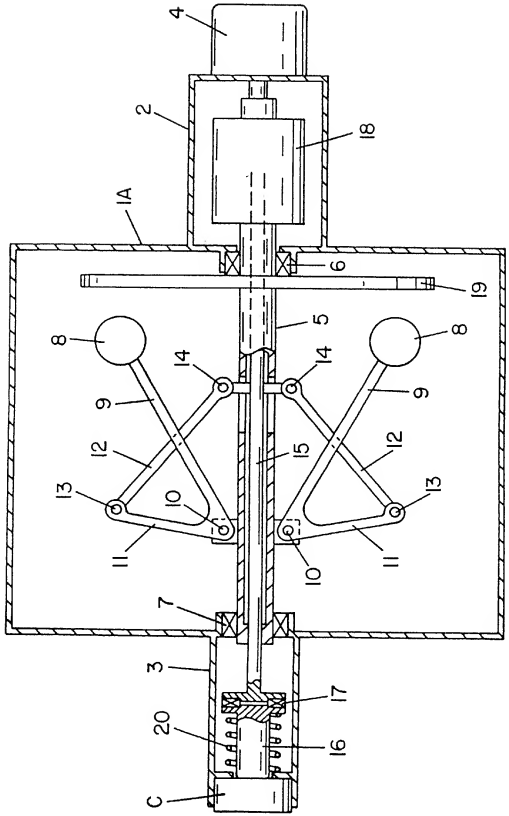

FIG. 2C

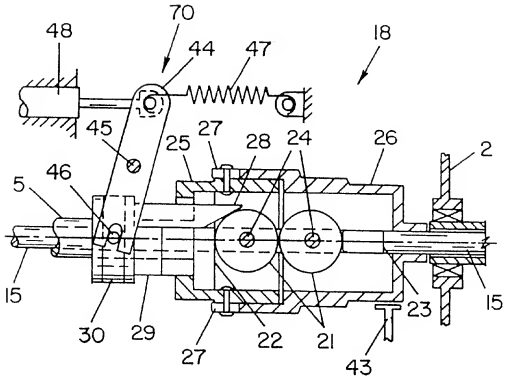
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FIG. 2D

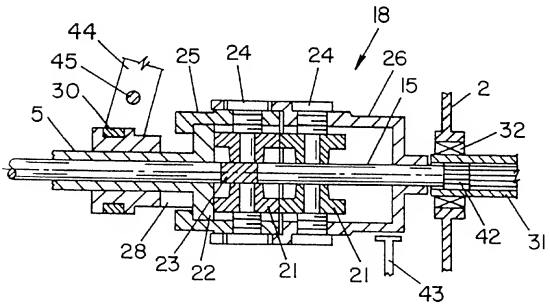
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FIG. 2E

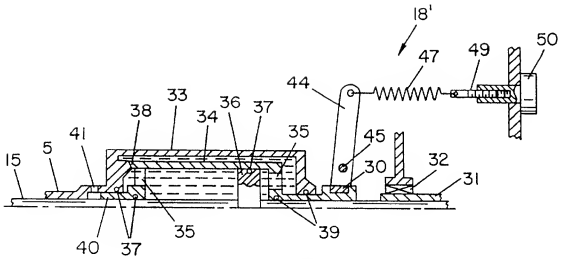
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FIG. 2F

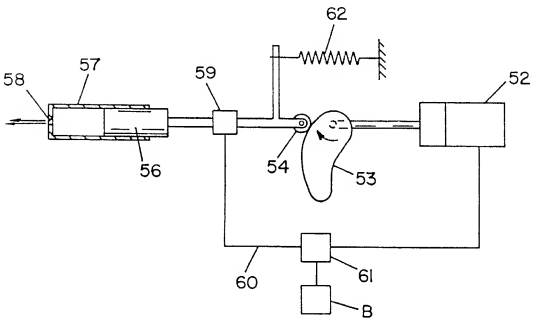
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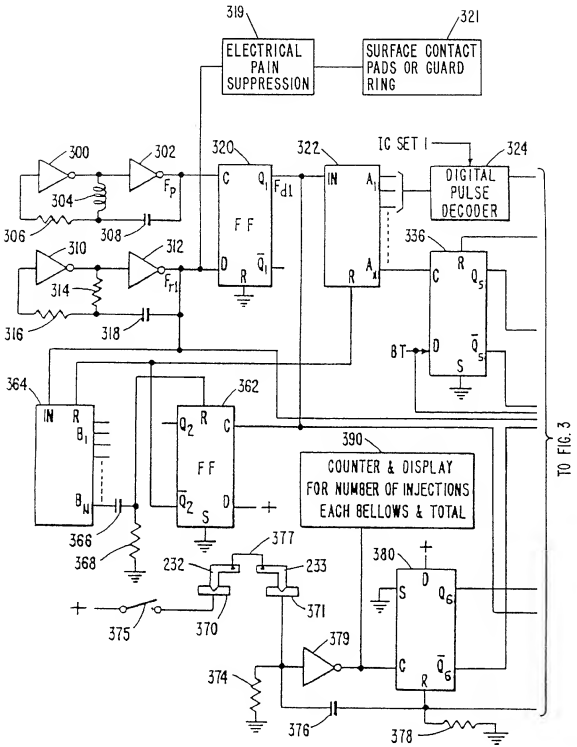
FIG. 2G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ELECTRICAL PAIN SUPPRESSION
321
SURFACE CONTACT PADS OR GUARD RING
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IC SET I
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DIGITAL PULSE DECODER
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8T
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COUNTER & DISPLAY FOR NUMBER OF INJECTIONS EACH BELLOWS & TOTAL
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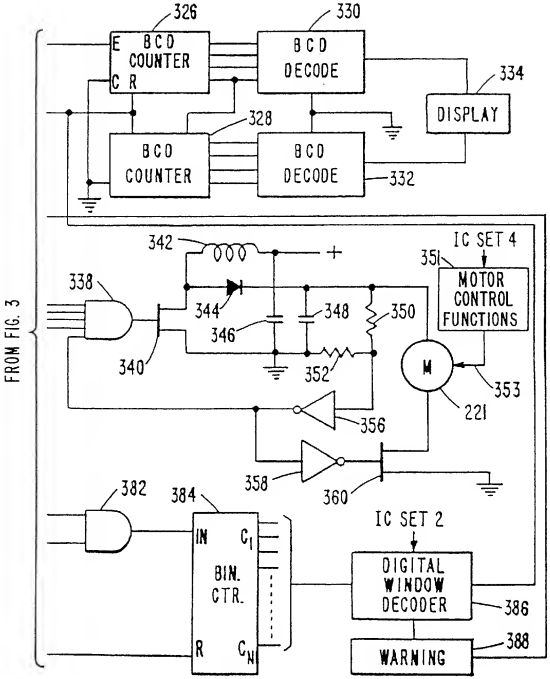
TO FIG. 3

FIG. 3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FROM FIG. 3

FIG. 3

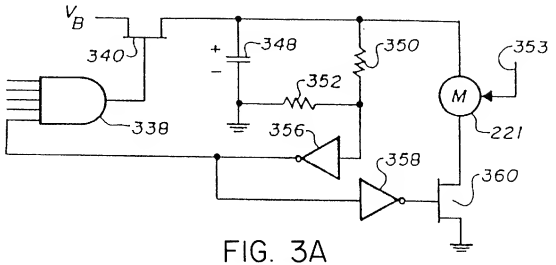


FIG. 3A

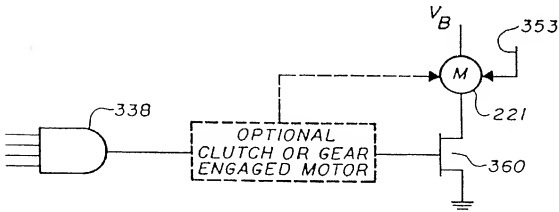


FIG. 3B

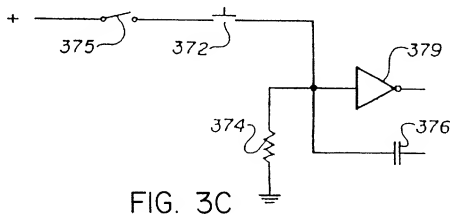
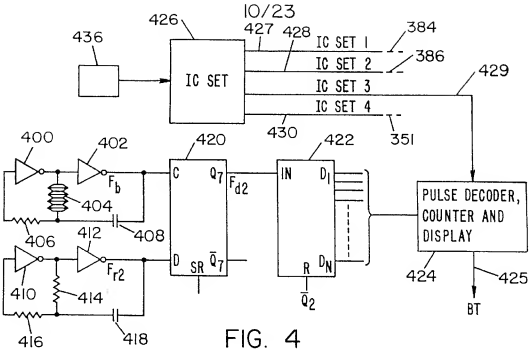


FIG. 3C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414
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C
Q7
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D1
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SR
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PULSE DECODER, COUNTER AND DISPLAY
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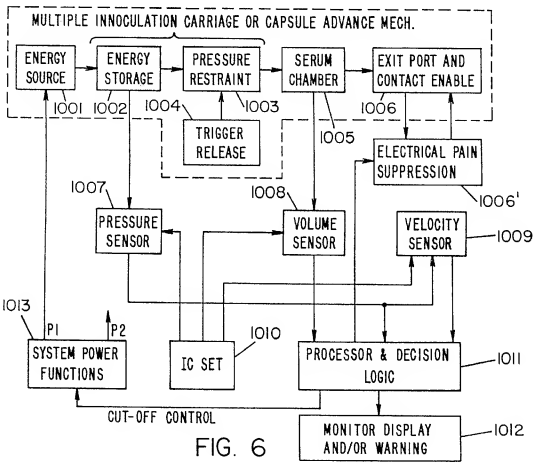
BT

FIG. 4


MULTIPLE INNOCULATION CARRIAGE OR CAPSULE ADVANCE MECH.
ENERGY SOURCE
ENERGY STORAGE
PRESSURE RESTRAINT
SERUM CHAMBER
EXIT PORT AND CONTACT ENABLE
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1002
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1003
1006
TRIGGER RELEASE
1005
ELECTRICAL PAIN SUPPRESSION
1006'
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1008
PRESSURE SENSOR
VOLUME SENSOR
VELOCITY SENSOR
1009
1013
P1
P2
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SYSTEM POWER FUNCTIONS
IC SET
PROCESSOR & DECISION LOGIC
1011
CUT-OFF CONTROL
MONITOR DISPLAY AND/OR WARNING
1012

FIG. 6

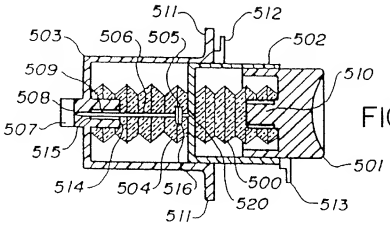

FIG. 5A

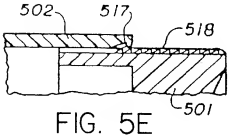

FIG. 5E

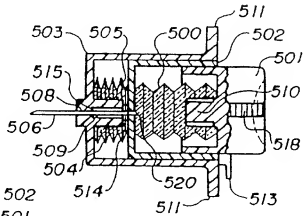

FIG. 5B

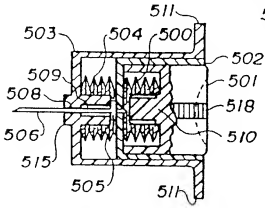

FIG. 5C

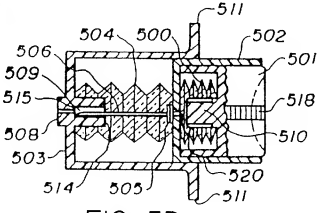

FIG. 5D

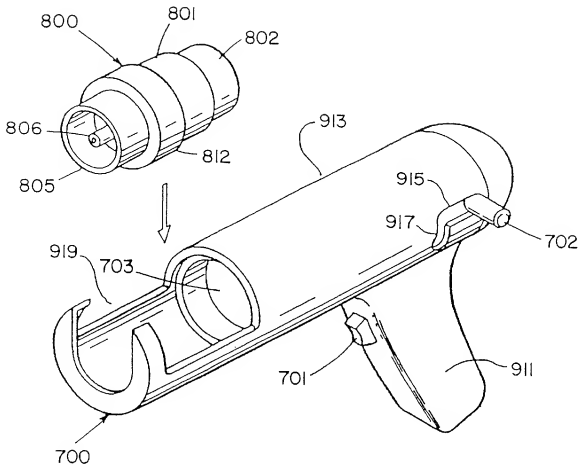

FIG. 7

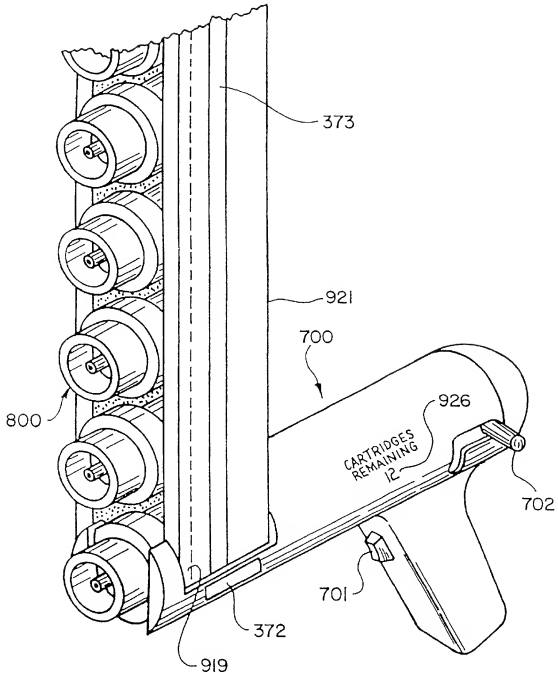

FIG. 7A

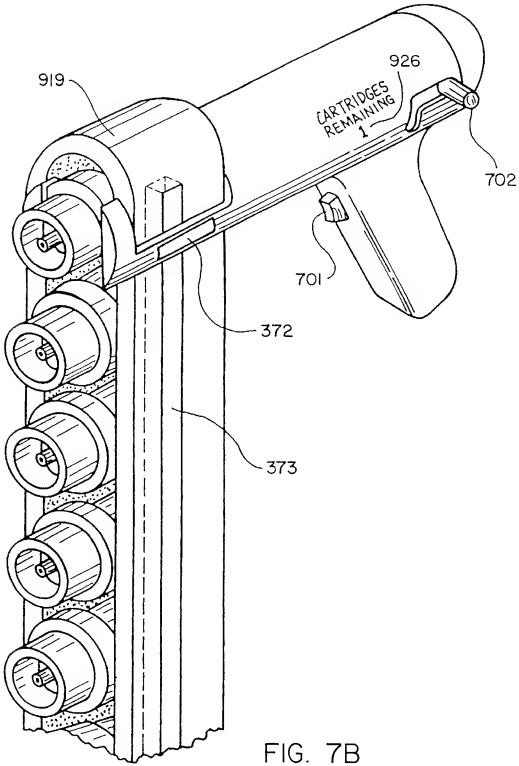

FIG. 7B

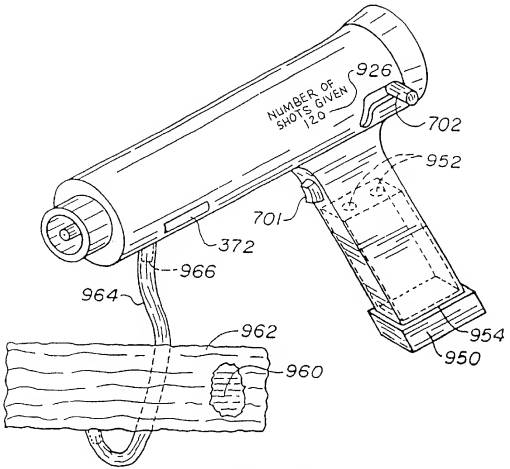

FIG. 7C

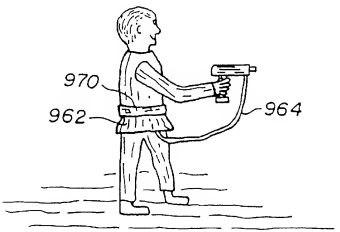

FIG. 7CC

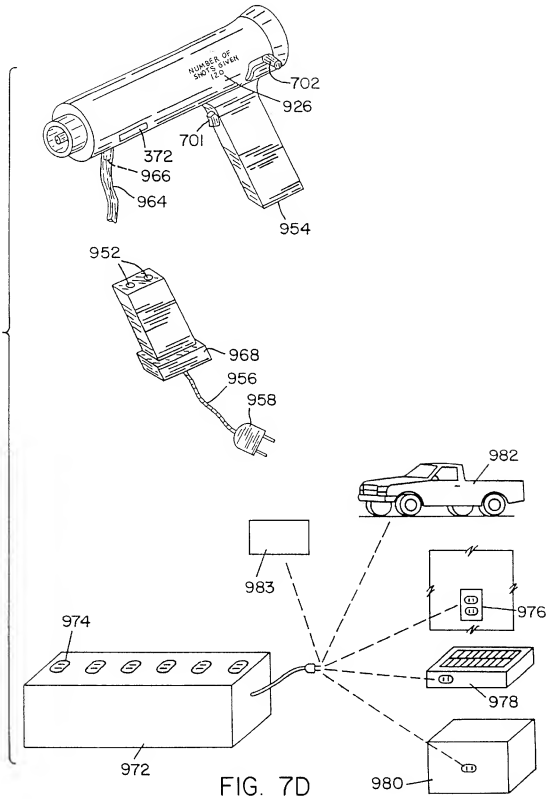
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FIG. 7D

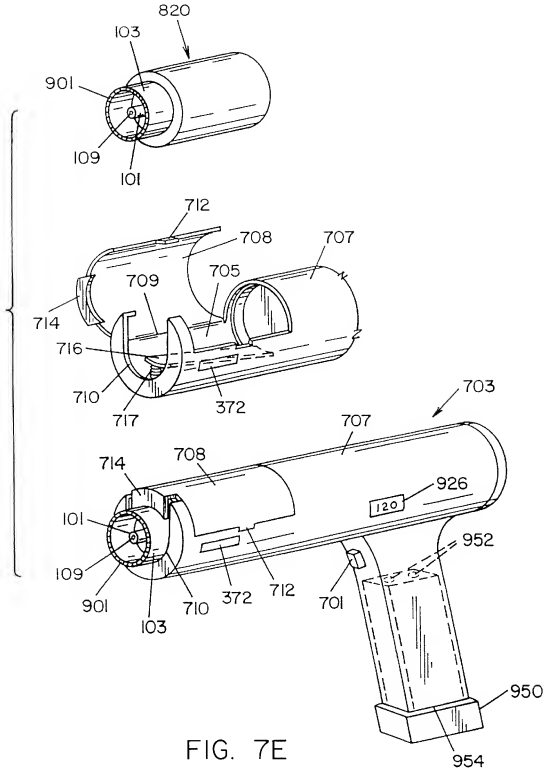
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FIG. 7E

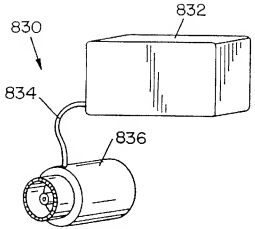

FIG. 7F

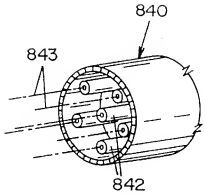

FIG. 7G

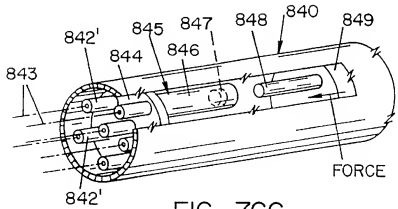

FIG. 7GG

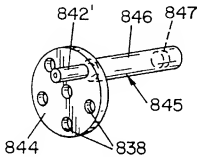

FIG. 7GG'

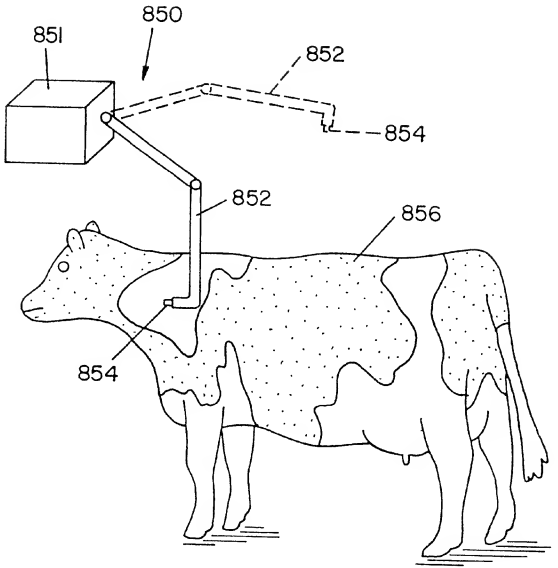

FIG. 7H

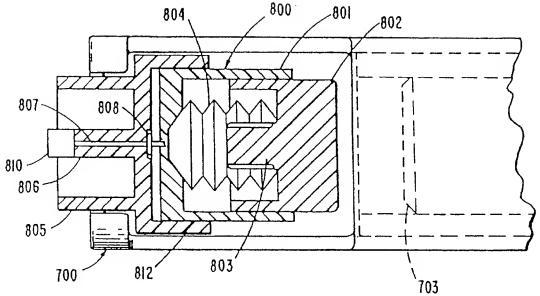
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FIG. 8A

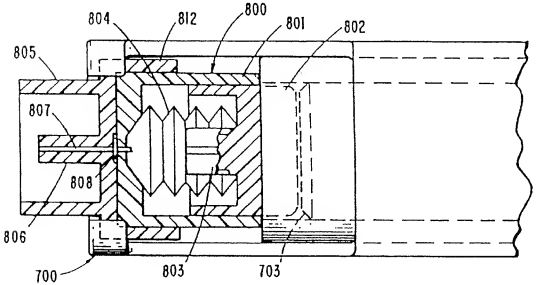
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FIG. 8B

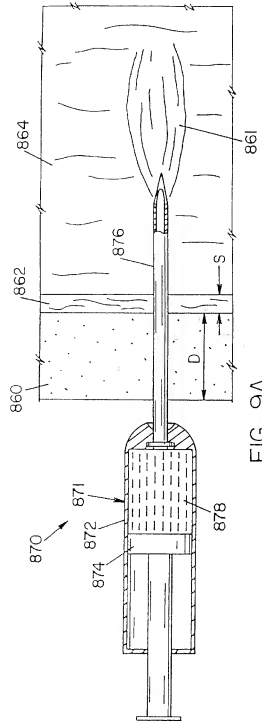

FIG. 9A
(PRIOR ART)

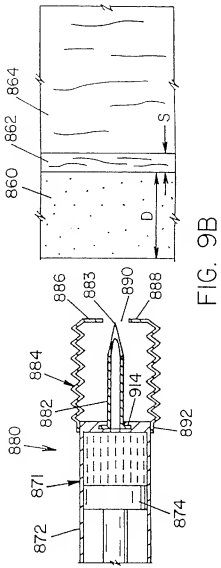

FIG. 9B

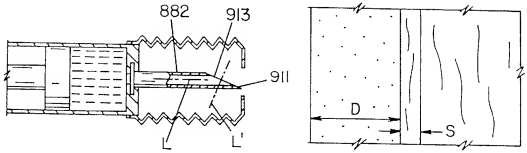

FIG. 9BB

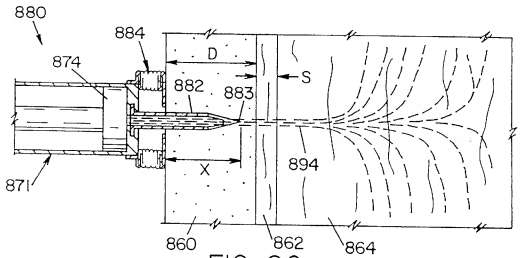

FIG. 9C

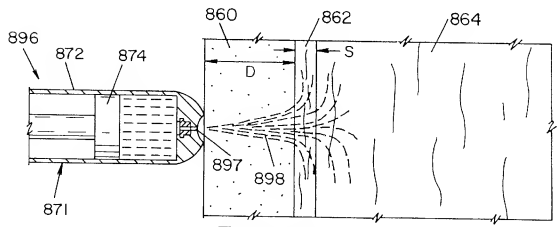

FIG. 9D
(PRIOR ART)

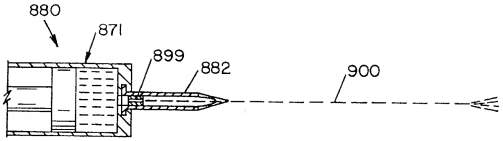

FIG. 9E

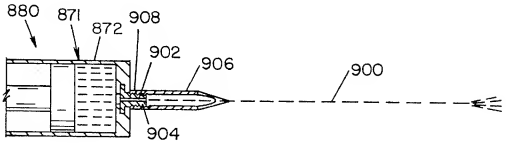

FIG. 9F

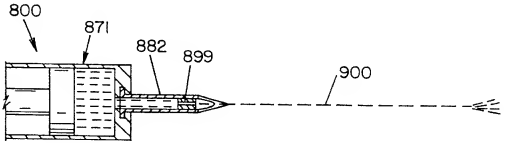

FIG. 9G

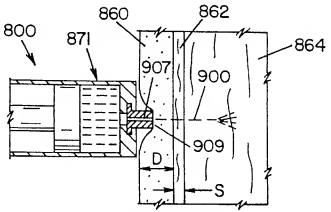

FIG. 9H

HYPODERMIC FLUID DISPENSER

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of corresponding U.S. patent application Ser. No. 08/253,416, filed Jun. 3, 1994 now U.S. Pat. No. 5,569,190, which is a continuation-in-part of Ser. No. 07/818,235, filed Jan. 8, 1992 now U.S. Pat. No. 5,318,522, which is a continuation-in-part of Ser. No. 07/336,636, filed Apr. 7, 1989 now U.S. Pat. No. 5,080,648, which is a file wrapper continuation of U.S. Ser. No. 07/059,620, filed Jun. 8, 1987, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to apparatus for the hypodermic injection of fluids.

2. Description of the Prior Art

Hypodermic fluid dispensers fall into two broad categories, namely, needle hypodermic dispensers and needle-less injectors, such as jet injectors. In the past, jet injectors have been particularly useful in large scale immunization programs, such as those administered by the World Health Organization (WHO) or the military, for example, where the number of subjects to be immunized in a single session is very large (more than 200 injections per session). However, in recent years, jet injectors are also finding benefit in more routine immunizations at WHO health centers around the world, where the number of subjects injected is relatively small at less than 40 injections per session.

In general, jet inoculation, as compared to needle inoculation, is less traumatic, requires less operator training, and allows a higher number of procedures per unit of time.

Although both needle hypodermic injectors and high pressure jet injectors have been widely used, the presently known devices have serious disadvantages with respect to the injection of humans and animals alike. There exists a high level of belief that conventional jet injectors are capable of cross-infection when the same orifice is used for subsequent injections, and the problems associated with needle injectors in the spread of AIDS (Acquired Immune Deficiency Syndrome) alone is sufficient motivation to avoid proliferation of today's needle systems. People cannot be relied upon to dispose of needle injectors in accordance with instructions and good practice, and an element of the population are tempted to reuse needles without knowledge of or regard for safe practices.

There are other important shortcomings in the present state of the art with respect to the inoculation of humans and large animals, such as cattle and pigs. In the case of large animals, needles used for injecting vaccines, vitamins, medications and hormones in large, food-producing animals often cause damage to prime portions of the meat through the creation of abscesses and scars, resulting in serious economic loss to the industry. In the majority of cases, these factors are even more prevalent because the needle injectors become dull or bent when used for multiple injections, which is a common practice in the industry. In addition, due to increased awareness of cross-infection in food-producing animals, agricultural leaders recommend that needles no longer be reused for animal injections. Furthermore, it is not at all unusual for multiple injections to be scheduled within the same period of time, this being true for humans and animals as well. However, repeated injections are usually

met with greater resistance by the recipient and also makes the procedure far more expensive. Hence, other important shortcomings would be overcome if more than one material could be injected at the same time.

Properly configured jet injectors provide the best chance for reducing the problems of cross-contamination, as well as the case where more than one injection is required at the same time, and could reduce the time to train people involved in the injection process. If jet injectors were effective for both animals and humans, a higher number of procedures per unit of time would be possible. The higher number of procedures is particularly important in the beef and dairy industry where each day a certain number of animals receive injections. This is also true for the multiple vaccine requirement for children.

Large animal hide, such as cowhide, can be very thick, often in the range of ⅛ inch to ⅜ inch or more in thickness. The hide includes the hair, a tough outer layer, and thereafter the dermis, whose inside surface has a rubber-like interface that begins the subcutaneous ("sub-Q") layer that separates the hide and the muscle. In cattle, injections are usually given in the neck, a leg or a hind quarter. A known CO_2-driven jet injector was experimentally tested on a freshly euthanized cow and the injection site was immediately followed by a pathologist's examination. It was found that the injection rarely penetrated the desired depth into the muscle.

Electrically operated needle-less injectors, previously disclosed, often require batteries, since standard power sources are not available at remote or isolated areas where injections are often given. People responsible for the immunization programs at these locations are concerned about disposal of these batteries, leading to the recommendation that rechargeable batteries be used. Indeed, the use of any electrically-powered injector used away from standard power sources would be enhanced if there were no batteries to be disposed of, yet sufficient electrical power were available.

The exit path of needle-less injectors, referred to as "orifices," present additional problems. Orifices are commonly found in the range of 0.004 to 0.014 inches. Extensive experimentation has shown that these orifices are likely to be ineffective for deep injections unless virtually perfect in structure and optimized in diameter for that particular injection site. If the orifices are poorly configured, they will fail to penetrate the thick hide of an animal, and satisfactory deep injections will not occur no matter what pressure is applied on the serum or other product being injected. When poorly configured orifices with diameters in tile range of 0.004 to 0.014 inches are used with injection pressures as high as 2,000 to 10,000 psi, effective deep injections have not been possible.

It is important that the speed at which needle-less injections are made is high, whereby the entire dose, or doses, enters the animal's body quickly by way of the high velocity jet stream. Movement could cause a loss of the initial penetration point and the injection could fail. As noted above with respect to cows, the injection must clear a hide thickness of ⅜ inch, or more in some cases, to achieve intramuscular ("IM") injection. Speed of injection, orifice size, quality of the jet stream, and lack of movement are necessary to get good IM injection results. If any of these characteristics are not fully met, or if the jet stream breaks down when the injection material is designed for an IM injection, the fluid will remain in the dermis, or in the subcutaneous layer instead, and could result in a less-effective or even a totally useless injection.

Presently known collapsible bodies or containers (generally referred to hereinafter as "containers") for holding hypodermic fluid do not readily provide the necessary fluid even when sufficient pressure is applied to the collapsible container. Collapsible containers do not exist for effecting the proper mixing of ingredients at the time sufficient pressure is applied to the collapsible container.

Present needle-less jet injector systems are very expensive. For one thing, these systems draw the serum or other injecting fluid into disposable capsules having walls thick enough to withstand the high pressures of about 1,700 psi or more for human IM injections.

Another problem with jet injector systems is that they "gum up" from the injectant material if the user fails to thoroughly clean the device on a timely basis. In some situations, such as field use with cattle, this procedure is difficult, if not impossible, in terms of available time and equipment.

Some injection fluids have higher viscosities than water, such as the BST growth hormone, Posilac, for enhancing milk production. If the fluid becomes too viscous because of lower temperatures, proper injection would not be possible. Presently, no temperature measuring systems for injection fluids are available.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an improved injection system for injecting materials such as vaccines, sera, growth hormones, vitamins, nutrients, and the like into a living body.

It is another object of the invention to provide a system for injecting material of the foregoing type into a body to obtain an effective injection even though the body may move somewhat during the injection process.

Yet another object of the invention is to provide a system for effectively injecting materials of the foregoing type through thick outer layers of a body and evenly disbursing the fluid from a high velocity, piercing jet stream at the inner regions in the event this is the desired target area.

It is a more particular object of the present invention to provide an injection system for injecting material of the foregoing type into large animals, such as cattle, having thick hides.

Another object is to provide an improved collapsible container or containers for holding material to be injected into an animal or person, once sufficient pressure is applied to the collapsible container or containers.

Yet another object is to provide an improved collapsible container or containers for holding injectant material which can bring together different ingredients to be injected once sufficient pressure is applied to the collapsible container or containers.

It is still another object to provide a jet injection system for injecting the foregoing types of materials into animal and human bodies having low-cost capsules with thin-walled cavities for holding the material to be injected.

Another object is to provide an effective injection system having multiple orifices for effectively reducing the time required for injecting a larger dose of material into animals and humans or for injecting multiple products at the same time.

Another object is to provide means to deposit each of the multiple products at different locations and/or at different injection depths.

It is still another object to provide means to inhibit injection site pain for the injection of singular or multiple products.

Another object is to provide electrical stimulation to dull injection site pain.

A further object is the provision of an injection system for preventing actuation of the system until certain conditions are met.

Another related object is to provide an injection system having a trigger which is actuable only when an anti-splash guard ring is properly in contact with the surface through which the injection is to be made.

Another object of the invention is to automatically provide an injection site mark or legend as an indicator that a group of animals or humans have already received a particular injection.

It is an additional object to prevent the unauthorized use of an injection apparatus.

Another more particular object is to provide an injection system having a trigger which is actuable only when an access code is first keyed into the system.

It is yet another object of the invention to provide a jet injection system having a hybrid needle/orifice combination which is designed to proceed through the outer layer of a body and obtain a more effective dispersion of the injectant material at a preferred injection site.

A further object is the provision of a hybrid/orifice combination jet injection system for animals which is so constructed that the system will not be impaired even if the animal moves during the injection process.

It is also an object of the present invention to provide an injection system having batteries or a storage capacitor which can be recharged with solar energy.

Another object of the invention is to provide a solar powered jet injection system and a solar generator for powering the jet injector at remote locations away from access to central electric power supplies.

Another object is to provide a light weight, easy to handle, injection system to allow for injections at a significant distance from an animal when close proximity is difficult to realize.

It is still another object of the present invention to provide a robotic injection system for the automatic injection of subjects such as the living bodies of humans and animals.

An additional object of the present invention is to provide an injection system with a replaceable component for connecting the system to a reservoir.

A further and related object is the provision of an injection system with a replaceable component for connection to an injection chamber and integral orifice.

Another object is to provide an injection system for mixing lyophilized ingredients at the time of an injection.

It is yet another object of the present invention to provide an injection system having a replaceable exit channel, orifice device, and/or a replaceable needle device, used in connection with a permanent medication chamber.

It is still a further object of the present invention to provide a temperature monitor for measuring the temperature of materials to be injected.

Another related object is the provision of a temperature monitoring device for an injection system for disabling the injection system if the temperature drops below threshold value(s).

A general object of the present invention is to provide an efficient, effective, yet economical system for injecting animals and people.

Other objects will become apparent from the description to follow and from the appended claims.

One form of the present invention makes possible the quick completion of the injection process through the hide of an animal. This form comprises a short perforating member with a jet system for forcing an injecting fluid under high pressure through the perforating member. Depending on the location of the injection, the perforating member of this hybrid combination is from ⅛ to ⅓ inch or more in length and is surgically sharp with a slanted point for easy insertion. When the perforating member is pressed into the hide of an animal, it creates an anchor point therefore eliminating the detrimental risk of movement by either the person making the injection or by the animal. The high pressure, high velocity jet stream from this vantage point is not deflected by the hair on the hide or the tough outer layer so that the jet flow remains coherent. The sharp, slanted needle point moves the debris aside rather than forcing it into the hide. Since the perforating portion of the hybrid combination extends through the hide, less injection pressure is necessary to reach the subcutaneous layer or intramuscular region of the animal if this is the injection site of choice. Consequently, lower motor torque is required, thereby allowing for a smaller power source for an equal number of shots than required by prior art systems. In addition, the orifice portion of the hybrid combination can be increased in size to provide for a faster discharge of the injection material thus allowing for larger doses with the same injection time. The lower cost motor and power source, along with a smaller and lighter weight for the system render it more economical and easier to use.

Because of the low cost, the hybrid perforator/orifice combination can be discarded after each use, further eliminating cross-contamination. Another embodiment of the invention incorporates a protective containment chamber into which the hybrid perforator can be withdrawn after the injection is completed, making the needle portion inaccessible for subsequent reuse, providing yet another way for preventing cross-contamination. When a jet injection is given through the perforating member, the orifice portion of the combination generates a coherent, evenly dispersed jet stream without having the needle portion penetrate the muscle, and thereby avoid the large economic loss in the meat industry from prior art needle injections.

Another aspect of the present invention is the provision of a collapsible container for holding one or more materials to be injected, having a needle device for injecting the material once collapsing pressure is applied to the container to force the materials from the container.

A further version of the invention is the provision of a collapsible container for a hypodermic system having holding containers or reservoirs (hereinafter generally referred to as "reservoirs") for ingredients to be combined for injection, and a needle device for penetrating a reservoir when sufficient pressure is applied to the collapsible container to cause the ingredients to be combined.

Another form of the present invention enables the use of low-cost, thin-walled capsules for holding the materials to be injected, even though the capsules must be able to withstand pressures commonly used with jet injection procedures. According to this version of the invention, the thin-walled capsule is held in a restraining structure, similar to a gun barrel, which serves to protect the capsule from expanding, bursting, or even leaking, as a result of the high pressure. The use of a restraining structure with a thin-walled capsule enables the mass immunization of animals and humans at a cost no greater than that experienced with conventional needle/syringe systems, but without all of the risks associated with needles. A restraining structure and thin-walled capsule eliminates the requirement for conventional needle injections and also eliminates the high cost of the thick-walled capsule of prior art systems. The hybrid perforator/orifice concept can also be used with the restraining structure and thin-walled capsule for higher pressure injections into thick hided animals, thus realizing an economical approach for this application as well.

Another aspect of the present invention is a jet injector system with multiple orifices for enabling fast injections of high doses of injecting material than heretofore possible. The number of orifices generally equals the number of times faster that an injection can be made. Multiple orifices could be used in human surgery, such as for plastic surgery where a fairly large surface pattern must be anesthetized, or in combination with multiple capsules to administer more than one serum product simultaneously at adjacent points of entry and, if advantageous, to further discourage the mixing of products at the injection site by providing for different injection depths for each product at each point of entry.

To better suppress the possibility of pain, another aspect of the invention provides for electrical stimulation to desensitize the injection site. This technique is especially useful with the added risk of pain due to multiple, simultaneous points of entry with either high velocity jet streams or needles.

Still another version of the present invention disables the actuation of the inventive system until additional predetermined conditions are met. An embodiment of this version includes a second switch in series with the actuating member such as a trigger.

In order to eliminate the battery disposal problem which could accompany battery-powered jet injection systems at remote locations, another embodiment of the present invention utilizes methods of energizing the low power requirements of an electrical injector. One version of this embodiment incorporates a relatively small gel cell or fluid electrolyte battery connected to a single injector or multiple injectors with light weight, low cost extension wires. For example, multiple injectors could be operated at the same time at remote health centers dealing with epidemic immunization requirements. The power sources for the injectors would be rechargeable directly from the transport vehicles if available. Such recharging can also come from an inexpensive low-power solar charger or directly driven with a solar generator of greater capacity. These recharging systems pursuant to the invention can eliminate the need for grid power or the more expensive and generally unwieldy electrical generators, carbon dioxide tanks, hydraulics and breakable foot pedals.

Another version of the invention is a robotic system. Herds of cattle ordinarily pass through separating chutes when they enter the barn. Animals to be injected can be electronically identified and separated from the herd. Such an identification could be by means of tags with bar codes. The separated animals could be restrained, and a long arm of a robotically-operated jet injector would inject the restrained animals and then release them when completed, pursuant to this version of the invention.

In a related version to the long arm of the robotic system, a long-armed manual system is provided with the injection mechanism and a surface contact enabling head at the target end and an actuating trigger at the user end. This embodiment has utility for the injection of large animals such as pigs, since getting close to the animal is sometimes difficult and/or inconvenient. Such a system could allow for removal of the robotic arm and use in the manual mode. The extended

arm concept can be provided as a totally independent manual system.

Another aspect of the present invention comprises an automatic marking system. The automatic marking system is used with respect to large numbers of animals or humans in close proximity to each other. The system includes a nozzle adjacent to the injection nozzle. The marking system automatically marks the subject when an injection is given. The marker, which should be a non-toxic dye, is preferably such that its expected life does not exceed the time between injections. Different indicia, such as using more than one color of the dye, will make multiple injections distinguishable. In this way, the user is able to tell which of the animals and/or humans in a large, close proximity group have already been injected with a particular product so that none are missed and none are injected more than once.

Another version of the present invention involves the elimination of the "gumming-up" which frequently affects jet injectors if the user fails to clean the device on a timely basis. According to this version of the invention, the jet injector gun has, as an integral unit for each serum to be administered, a tube for connection to the reservoir having the injectant material, a chamber and an orifice, which is inserted into the gun as a single composite system containing all of the elements necessary for delivering one or more serums at the same time. During a series of shots, the injectant fluid or fluids are drawn into the chamber or chambers and injections are given through the integrated orifice system. At the completion of the entire series of shots, the integral unit is removed and discarded. Only the removable and disposable integral unit contacts any injection fluid; the gun itself does not contact any fluid and does not gum-up. The system could be used with a single-shot disposable orifice and perforator for each fluid to eliminate cross-contamination.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated in the accompanying drawing in which:

FIGS. **1** and **1A** illustrate side, cross-sectional views of two multiple inoculation jet injectors in accordance with the invention;

FIG. **1B** is a partial pictorial view of a modified body ring guard for use in the injectors of FIGS. **1** and **1A**;

FIG. **1C** is a pictorial view of a modified nozzle for use in the injectors of FIGS. **1A** and **1B**;

FIGS. **2A** and **2AA** illustrate cross-sectional views of jet injector systems with the fresh injectors of FIGS. **1** and **1A**, respectively, installed therein;

FIGS. **2B** and **2BB** illustrate side, cross-sectional views of the jet injector systems of FIGS. **2A** and **2AA**, respectively, with the injectors partially expended;

FIG. **2C** is a schematic diagram illustrating a fly-wheel system as an alternative to the spring of FIG. **2A**, **2AA**, **2B** and **2BB** for producing injection pressure;

FIG. **2D** shows a cross-section of a mechanical latch when the flyweights of FIG. **2C** are in the most inward position;

FIG. **2E** shows the latch of FIG. **2D** in final release, when the flyweights are in the most outward position;

FIG. **2F** shows a rotating hydraulic latch in the locked position;

FIG. **2G** is a schematic diagram of apparatus for the direct drive of the forces for an injection procedure.

FIG. **3** and FIG. **4** are schematic representations of one embodiment of the control, monitoring, and motor drive circuitry of the jet injector system of FIGS. **2** or **7**;

FIGS. **3A** and **3B** are schematic representations of alternative embodiments for the motor drive circuitry in the jet injector system of FIGS. **2** and **7**;

FIG. **3C** is an alternate embodiment of the power switch shown in FIG. **3**;

FIGS. **5A** through **5E** illustrate side, cross-sectional views of a needle hypodermic injector in accordance with another embodiment of the invention;

FIG. **6** is a block diagram overview of a multiple inoculation jet injector system in accordance with another embodiment of the invention;

FIG. **7** is a pictorial view of another embodiment of a jet injector in accordance with my invention;

FIG. **7A** shows an oversized perspective view of the injector shown in FIG. **7**, with a fresh magazine with N unused injections positioned in the dispenser;

FIG. **7B** shows the oversized magazine illustrated in FIG. **7A** with the injections having been used;

FIG. **7C** is a pictorial view of the jet injector wherein the rechargeable power source is a removable module and a multi-dose container is connected to the injection head to permit the rapid continuation of an injection procedure for a large number of subjects;

FIG. **7CC** is a pictorial view of the embodiment shown in FIG. **7C** in use;

FIG. **7D** shows the format of another embodiment of the invention showing an injector capable of being driven by alternate sources of electrical power;

FIG. **7E** is an exploded pictorial view of a jet injector system according to another embodiment of the invention, using thin-walled capsules in a high pressure injection chamber;

FIG. **7F** is a pictorial view of a disposable, anti-gumming sub-system according to another embodiment of the invention, having a replaceable serum container, connecting hose, injection chamber and exit orifice as a single unit;

FIG. **7G** is a pictorial, partial view showing a multitude of exit orifices from a single injection chamber;

FIG. **7GG** is a pictorial illustration of another embodiment of the invention showing multiple independent injection chambers each having its own exit orifice;

FIG. **7GG'** is a detailed, pictorial view of a single injection chamber and exit orifice from the multiple chambered system of FIG. **7GG**;

FIG. **7H** is a pictorial view of a cow receiving a jet injection with a robotic arm according to another embodiment of the invention;

FIG. **8A** shows a side, partial view of the jet injector system illustrated in FIG. **7** with a fresh cartridge installed therein;

FIG. **8B** shows a side, partial view of the jet injector system of FIG. **7** in which the cartridge has been breached;

FIG. **9A** is a schematic showing the "pooling" effect when the injectant is introduced into the injection site using the prior art needle and syringe occurring after an injection;

FIG. **9B** is a schematic illustrating an encapsulated perforator prior to insertion, according to another embodiment of the invention;

FIG. **9BB** is a side view of a perforator with a slanted tip according to an embodiment of the invention;

FIG. **9C** shows in schematic the perforator of FIG. **9B** inserted in the hide of an animal with a protective shield fully compressed;

FIG. **9**D shows in schematic form an injection system with jet stream degradation when the entrance point for the jet stream is the outer surface of the hide, under a prior art system;

FIG. **9**E is a schematic view of a perforator with a conventional jet orifice located at the input channel of the perforator;

FIG. **9**F is a schematic view of an embodiment of the invention showing a permanent jet orifice with a removable perforator;

FIG. **9**G shows schematically the jet orifice located at the exit end of the perforator pursuant to another embodiment of the invention; and

FIG. **9**H shows a schematic view of a very short, flat, non-sharpened tubular orifice serving to stretch and detent the injection site in preparation for the high velocity jet stream according to a further embodiment of the invention.

DETAILED DESCRIPTION OF THE EMBODIMENT

FIG. **1** illustrates a liquid filled jet injector or dispenser **1** stored in a protective cover **110**. The jet injector of FIG. **1** comprises a liquid-filled bellows **100**; a front plate **102** which comprises a pair of guide rod recesses **111** and **112**, a set of thin washers **116** which have an inner diameter slightly larger than the minor diameter of bellows **100**, a pair of holes **117** in each of the washers **116** that are aligned with the guide rod recesses **111** and **112** and with the openings **105** and **106**; a spacing guard ring **103**, with an interrupted pattern **901** shown in FIG. **1**B to prevent sliding between the dispenser and the skin; a disposable and replaceable exit nozzle **101** with an output port **109** (shown in greater detail in FIG. **1**C); a ram **113** with a groove **114**; and a back plate **104**. Guard ring **103** can advantageously have the features shown in FIGS. **1**B and **1**C, respectively. An interrupted pattern **901** at the end of ring **103** in FIG. **1**B prevents sliding between the dispenser and the skin of the person being injected. The nozzle **101** has its end **903** threaded in FIG. **1**C or the like so that it can be disposable and replaced. The back plate **104** comprises openings **105** and **106**, aligned with holes **117** in washers **116**, which accept a pair of guide rods when the jet injector cartridge is installed in the system of FIGS. **2**A and **2**B; and a ring **115** which forms the recess **107** for receiving a drive spring **227** of the system of FIG. **2**A. The injector of FIG. **1** may be a disposable injector or a multitude of permanent reusable injectors for the administration of, for example, the growth hormone. The attendant can pre-fill a multitude of the cartridges shown in FIG. **1**A carrying them in a belt holster, or as shown in FIGS. **7**C and **7**D, in a single large container or sack with a hose connected to the injection chamber. The output port can be made of ceramic, plastic, glass or metal and may be removable and replaceable or a permanent part of the injection head. In either case, device economy is improved by fabricating a molded output port having tile flow orifice formed by first inserting a preformed metal, or other sufficiently sturdy member, whose size and shape, i.e., length, diameter and flow path angilation are chosen to provide laminar flow. Some of the other possible methods for forming the orifice include laser boring, water jet cutting and electron beam cutting.

FIG. **2**A is a cross-sectional representation of an illustrative embodiment of a hypodermic jet injector system with a fresh jet injector of FIG. **1** installed therein. The system of FIG. **2** in general comprises: a housing **220**; the control, monitoring and display arrangements of FIGS. **3** and **4** (not shown in FIG. **2**A); an electric drive motor **221**; a motor output shaft **222** with a threaded portion **223**; a loading ram **224** with internal threads **225** which mate with the threads **223**; an energy storage spring **227**; a reluctance transducer shield **226** to be described with respect to FIG. **3** later herein; a pair of guide rods **228** and **229** that serve to align and support the bellows **100**; retaining latches **238** and **239**; and a trigger mechanism which comprises the detents **232** and **233**, the follower springs **230** and **231** and the follower blocks **234** and **235**. The mechanism for releasing the detents **232** and **233** is not shown in FIG. **2** and any suitable mechanical linkage which effects the simultaneous lifting of the detents **232** and **233** is satisfactory. In an alternative mechanical embodiment for loading energy storage spring **227**, threaded motor shaft **222** is replaced with a shaft driven cam which serves to compress energy storage spring **227** as the motor shaft rotates.

An alternate embodiment of the injector of FIGS. **1**, **2**A and **2**B is illustrated in FIGS. **1**A, **2**AA, and **2**BB. In the alternate embodiment, the liquid-filled bellows **100** is replaced by a collapsible liquid-filled "hat" diaphragm-type structure. Detents **111** and **112** of FIG. **1** are replaced in FIG. **1**A by slot **120** for mounting and firmly securing hat structure **122** to the front plate **102**. This arrangement is equally effective if the fluid chamber is replaced by a piston-type syringe.

FIG. **2**AA is a cross-sectional representation of the injector system with a fresh injector installed therein. When the diaphragm **122** is inserted in the now conformal housing **220**, a slight initial collapse of **122** occurs to facilitate chamber venting and to assure an effective inner folding action as the multiple injections follow. The conformal shape of housing **220** constrains fluid chamber **122** and thus prevents undesired outward expansion under the influence of the injection pressure when the spring **227** is released. Back plate **124** is configured to assure that the detents **232** and **233** do not interfere with fluid chamber **122** as it progressively folds inside its outside diameter (FIG. **2**BB) with each additional injection. In the case of the syringe, the piston is progressively pushed to the right as the injections occur.

While the following discussion is specific to the embodiment of FIGS. **1**, **2**A and **2**B, the discussion applies equally to the alternate embodiment of FIGS. **1**A, **2**AA and **2**BB and the piston-type syringe dispenser.

Prior to the time that a fresh jet injector of FIG. **1** is installed in the system of FIG. **2**, a manually operable reset switch **436** of FIG. **4** is operated to enable the IC Set circuit **426** of FIG. **4** to establish initial system conditions. The output signal of the circuit **426** enables motor control functions **351** of FIG. **3** to draw the loading ram **224** back into the initial position illustrated in FIG. **2**A or to rotate the cam to its starting position; output signal **427** initializes the digital pulse decoder **324**; output signal **428** is transmitted to the digital window decoder **386**; and signal **429** is transmitted to the pulse decoder, counter, and display **424**. Alternatively, the ram **224** or the cam can be manually returned to the initial position at the time that a fresh bellows is installed.

The functions of the pulse decoder **324**, the window decoder **386** and the pulse decoder counter and display **424** will be apparent from the description of FIGS. **3** and **4** which appears later herein. After the system is thus conditioned, a fresh jet injector is removed from the protective cover **110** and inserted into the housing **220** as illustrated in FIG. **2**A. To install the jet injector, the guide rods **228** and **229** are inserted into the openings **105** and **106** in the back plate **104**,

through the openings **117** in the washers **116**, and the injector is moved into the housing **220** until the front face of the front plate **102** is clamped by the latches **238** and **239**. As the jet injector is moved into the housing **220**, the detents **232** and **233** engage the notches **108** in the back plate **104** and follow the motion of the back plate as it is moved to the left in FIG. **2**A. The follower blocks **234** and **235** follow the motion of the detents **232** and **233** to the left and thus compress the follower coil springs **230** and **231**. Further, when a fresh injector is in position as illustrated in FIG. **2**A, the energy storage spring **227** enters the recess **107** in the back side of the back plate **104**. The system of FIG. **2**A is in condition for an operator to perform a series of inoculations.

Under operator control, the system of FIGS. **2**A, **3** and **4** selectively provides power to the motor **221** to advance the ram **224** to the right in FIG. **2**A and thus compress the coil spring **227**. As described later herein, the circuitry of FIG. **3** monitors the degree of compression of the spring **227** and removes power from the motor when the compression reaches a target value or when the selected flywheel speed of FIG. **2**C is reached. The target value is either a default value established by the manufacturer or a value established by an operator on the basis of experience. After the spring has been compressed or motor speed reaches the target value, the guard **103** is held perpendicular to and against the skin at a suitable site of a subject to be injected. The outer ring surface of guard **103** may have a tooth-like pattern **901** to reduce the possibility of the ring sliding along the skin during the course of an injection. Guard ring **103** can also contain electrical pain suppression contacts. If the ring does slide during the injection the subject may receive a "jet cut" rather than a jet inoculation. The operator initiates injection by depressing a trigger, which as indicated earlier herein is not shown in the drawing. Depression of the trigger simultaneously releases the detents **232** and **233** from the recesses **108** in the back plate **104**. The energy stored in the spring **227** is released and the back plate **104** is rapidly driven to partially collapse the bellows **100**. As the bellows is collapsed, a desired amount of fluid is driven through the output port **109** in the projection **101**. Advantageously, the use of a compressed spring as a source of energy provides a high initial pressure which reduces as the bellows **100** collapses. The size and the length of the port **109** and the pressure profile supplied by the compressed spring projects the fluid with a desired pressure profile which assures hypodermic injection of the fluid to the desired depth. The diameter and the length of the port **109** are chosen to assure laminar flow of the fluid from the chamber to the output tip. As the bellows collapse, guide washers **116** move together and prevent the bellows from bending under the influence of the high initial force of the injection.

As explained earlier herein, when a fresh injector is inserted into the housing **220**, the follower springs **230** and **231** are compressed. Therefore, after the trigger is released, the follower blocks **234** and **235** and the detents **232** and **233** are driven to the right in FIG. **2**A until the detents again engage the corresponding recesses **108** in the back plate **104**. The follower blocks **234** and **235** may be connected to a small dashpot if a delay in this action is desired. The system of FIG. **2**A is then again ready for the operator to initiate another injection.

Because the wall of the bellows **100** has a finite thickness, a fully collapsed bellows has a substantial length. The length of the ram **113** approximates the length of the collapsed bellows. Without the ram **113**, valuable fluid is left in a fully collapsed bellows. The groove **114** in the ram **113** prevents the trapping of fluid in the portions of the bellows which surround the ram **113**.

Two additional alternatives to spring **227** can provide the pressure needed to give an effective injection. The first is a motor-generator in combination with an energy storage flywheel as shown in FIG. **2**C. This embodiment represents a device for another method of intermediate energy storage when the power source is insufficient for a direct drive injection, and is especially useful for realizing a compact, lightweight design, even when a much higher pressure is required for an effective injection. In the second embodiment shown in FIG. **2**D, injection pressure is developed directly from the motor with no intermediate storage of energy, such as a spring, compression chamber or flywheel. However, if applicable, an energy storage flywheel could be advantageously used with the drive mechanism shown.

The components of one version of a flywheel system of FIG. **2**C are the housing **1** for the flywheel with rear extension **2** and front extension **3** to enclose the other moving parts. A motor **4** capable of accelerating to high speeds directly drives a spindle **5** rotating in a rear bearing **6** and a front bearing **7**. At least two flyweights **8** swing on arms **9** on a set of pivot bearings **10** fastened to spindle **5**. Arms **11** are rigidly attached to arms **9** and the flyweights **8**. The centrifugal load of flyweights **8** is transmitted from arms **11** to a pair of links **12** through a pair of pivot bearings **13**, then through a pair of pivot bearings **14** to a thrust rod **15**. Rod **15** rotates with the spindle **5**, and moves axially to the left in FIG. **2**C as the radial distance of the flyweights from the central axis increases. The thrust load is transmitted to a plunger or ram **16** which moves axially without rotating and provides the force required for operating an injection device. Thrust bearing **17** is therefore required for transmitting the load from rod **15** to plunger **16**. Plunger **16** transmits a pressure or load to a container C to effect the discharge of an injectant as a jet stream.

The reaction from thrust bearing **17** is exerted at bearing **6**, which must have a thrust capability equal to that of thrust bearing **17**. Ball or roller bearings, which can be used for these bearings, should have very low friction, but the energy loss can be substantial because of the high loads and speeds. A rotating latching device **18** which locks all of the rotating parts together can eliminate the thrust load losses during acceleration. The latch could operate directly on the flyweights or could latch thrust rod **15** to the spindle, as indicated in FIG. **2**D.

A constant-inertia wheel or flywheel **19** can be added for storing additional energy during acceleration that would compensate for thrust bearing and other losses during the injection phase, after latch release.

A light spring **20** restores the mechanism to the initial position after injection. It may be desirable to apply electrical or mechanical braking to stop flywheel rotation after injection. The energy loss from braking will be small since the speed is already greatly reduced.

The magnitude and variation of inertia, the characteristics of the flyweight-thrust rod linkage mechanism and the friction losses affect the force variations during injection. The linkage shown has an increasing mechanical advantage as the flyweights move outwardly, partially offsetting the decrease in centrifugal force from the speed drop caused by the increase in the moment of inertia and by the friction losses.

FIGS. **2**D, **2**E and **2**F show rotating latch designs. However, many parts required for assembly are not shown as individual items for reasons of clarity. While these figures are directed to a particular design concept, it should be noted that numerous methods of mechanical, hydraulic, magnetic

and electrical latching systems are applicable. For example, release of the hydraulic latch of FIG. **2**F can be facilitated with a variable force spring, wherein, as the motor accelerates to reach the selected speed, centrifugal force will increase accordingly. When centrifugal force finally exceeds the holding force of the variable tension spring, the latch will release. In another centrifugal force embodiment, the spring is replaced by holding magnets or, as an alternative, the flyweights themselves are magnetically held. In this case, break-away will occur when centrifugal force exceeds the holding force of the magnets. A variable holding force with the magnets can be achieved by changing the amount of overlap between the magnet face and the metal plate to which it is attached.

One embodiment of an electrical release that is applicable to FIGS. **2**D and **2**E utilizes a motor tachometer. In this case, the user will dial or key into the system the desired injection pressure, that is, motor RPM. When the target speed is achieved, an electrical signal is emitted to actuate a solenoid. Alternatively, a coil would around one or more holding magnets could be energized to momentarily reduce the holding force and a release will occur. Using an RPM dependent signal will assure a consistent release point and will avoid the possibility of release error due to variations that may occur in the non-electrical holding means described above.

Turning now to a detailed explanation of FIGS. **2**D, **2**E and **2**F, FIG. **2**D shows a mechanical latch in the position which locks thrust rod **15** to spindle **5**, when the flyweights are in the most inward position. FIG. **2**E shows the same latch in the final released position, when the flyweights are in the most outward position.

Two diametrically opposite pairs of rollers **21** roll on a flange **22** on spindle **5** and on two diametrically opposite lugs **23** on thrust rod **15**. A pair of axles **24** fastened to a pair of cylinders **25** and **26** hold the rollers in axial alignment. These cylinders **25**, **26** can turn freely on flange **22** and rod **15** but are held in axial alignment with each other by a set of keys **27**. The keys allow slight axial movement between cylinders **25**, **26**; therefore, the thrust load is transmitted through rollers **21** without putting any significant load on the axles.

Release is triggered by moving a pair of prys, wedges or inclined planes **28** axially to the right, forcing rollers **21** in cylinder **26** to roll off lugs **23**. The thrust load is then transmitted to plunger **16** (FIG. **2**C) and rod **15** moves axially to the left. Wedges **28** project from a ring **29** through slots in flange **22**. Ring **29** slides freely on spindle **5**, but is forced to rotate with the spindle by the wedges **28** in the slots. The trigger moves a non-rotating collar **30** and the wedges axially.

One end of latch **18** is supported when rod **15** slides in a rotating member, which in this case is a hollow drive shaft **31**, which turns rod **15** with keys or splines **42**. Shaft **31** is supported by a bearing **32**.

The direction of flywheel rotation would preferably be in the same direction in which cylinders **25** and **26** must rotate for release (i.e., clockwise in FIGS. **2**D and **2**E when viewed rightward from the left ends). Relatching can then be accomplished by momentarily retarding a cylinder with a brake **43** when starting, while the force from spring **20** exceeds the flywheel thrust and holds rod **15** against the right limit of its travel.

A trigger **70** comprises a yoke **44** which is pivoted on a pair of pins **45** in housing **2** and which contacts a pair of pins **46** on collar **30**. A bias spring **47** attached to trigger **70** and housing **2** maintains the locked position until release is triggered manually or by an electrical solenoid **48**, also attached to the trigger and to the housing.

FIG. **2**E shows rollers **21** as if they revolved 90 degrees from their position in FIG. **2**D, but the angle can be any value within a range set by the clearance between rollers **21** and lugs **23** and wedges **28**.

Features of this latch are that there are no highly-loaded bearings and no radially outward mass transfers, which would reduce speed without producing any useful thrust.

The need for a hollow keyed drive shaft **31** can be eliminated by a latch designed to operate at the left side of FIG. **2**C or by interchanging the positions of the motor and the injection device. The injection device must be designed for a pull, rather than a push, from the thrust rod in the latter case.

FIG. **2**F shows a rotating hydraulic latch **18'** in the locked position. A cylinder **33** at the end of spindle **5** is filled with hydraulic fluid F. An inner cylinder **34** contains fluid F which is at an elevated pressure in the locked position. Cylinder **34** has end walls with openings **35** through which fluid can flow freely. The pressure is produced by a piston **36** and rod **15**, which transmits the thrust load to fluid F. Seals **37** and a seat **38** prevents leakage. Part numbers correspond to those in FIGS. **2**D and **2**E where corresponding parts are employed.

Inner cylinder **34** is moved a small distance to the right by collar **30** to form an opening at seat **38** and release the pressure on the left side of piston **36**. The fluid flows between cylinders **33** and **34**, and flows into the increasing volume to the right of piston **36** as the piston moves to the left. The fluid volume within the latch remains constant. No flow to or from the outside is required. A pair of seals **39** complete the sealing of latch **18'** and are not exposed to high pressures. A projection **40** from inner cylinder **34**, with a vent **41** to the atmosphere, permits a constant volume system and balancing of the trigger load.

The diameters of piston **36** and seat **38** can be proportioned to produce a particular trigger force at a particular speed in either direction. If the force on collar **30**, originating with flyweights **8**, is to the right in FIG. **2**F, latch **18'** will trip automatically at a load set by bias spring **47**, a screw **49**, and an adjusting knob **50** in the housing. Another feature of latch **18'** is that it can easily be locked at any position of the flyweights and plunger **16** (FIG. **2**C) before acceleration begins. This facilitates injection of varying serum volumes from one size cylinder or capsule.

Fluid pressures in the latch can be much more moderate than the injection pressures if the latch diameters are sufficiently large.

In the direct drive system of FIG. **2**G, a high torque gearmotor **52** rotates a cam **53** (clockwise in FIG. **2**G) whose increasing radius directly drives a cam follower **54** which in turn transmits a drive force to compress a piston **56** in injection chamber **57** to expel the injectant through an exit port **58**. A force transducer **59** continuously detects the force exerted on piston **56** and generates a feedback signal **60** that increases or decreases drive power to the motor drive through a controller **61** powered by a battery B, in order to maintain the target pressure throughout the injection cycle. A return spring **62** pulls piston **56** back to its starting position when cam **53** rotates beyond the maximum stroke of the injection cycle. An optional motor-generator and constant inertia flywheel (not shown) is also applicable for energy storage if insufficient short term power is available for a direct drive system.

The cam follower **54** and piston **56** in FIG. **2**G could be replaced by other means for transmitting a drive force, and

piston **56** and injection chamber **57** could have other forms rather than the piston and cylinder as shown. Piston **56** could be other ram means and the injection chamber could be other containers from which the injectant is expelled.

The above description is a general outline of the inoculation process with detailed reference to the means for developing injection pressure, system control, monitoring, motor drive and display apparatus of FIGS. **3** and **4**. While it is our intent to provide safe, low cost and convenient-to-use hypodermic injectors, we also provide measures of electronic monitoring, motor drive and control not found in known jet injectors.

FIG. **6** provides a block diagram functional overview of a hypodermic jet injector system in accordance with my invention. The dotted line of FIG. **6** provides a logical division between the mechanical portions of the injector system and the electronic monitoring, motor drive and control portions of the system.

The functional boxes **1001** through **1006** of FIG. **6** correspond to elements of the illustrative embodiment of FIGS. **2**A and **2**B as follows:

FIG. 6	FIG. 2A or 2C
Energy Source 1001	Motor 221
Mechanical Energy Storage 1002	Spring 227 or flywheel 8
Pressure Restraint 1003	Detents 232 and 233 or latch 18
Trigger Release 1004	Not shown in drawing
Serum Chamber 1005	Jet injector of FIG. 1 including Bellows 100
Exit port and contact enable 1006 and electrical pain suppression 1006'	Output port 109 and guard ring 103

Although the illustrative embodiment of FIG. **2**A employs an electrically-operated geared down motor **221** to compress the coil spring **227**, this requirement can be fulfilled by a variety of manual arrangements utilizing gears or other means of mechanical advantage. While the spring is preferred for storing mechanical energy, the blocks **1001** and **1002** of FIG. **6** could be replaced by other arrangements, e.g., a powerful solenoid, gas, hydraulic pressure, the flywheel, or direct drive cam discussed earlier. The critical requirement of the blocks **1001** and **1002** is that the serum chamber **100** receives enough force for a sufficient period of time to assure an effective inoculation.

The monitoring functions of FIG. **6** inform an operator when the device is ready to perform an injection, i.e., all system parameters are within acceptable limits of performance. A warning is issued when performance is not within limits and the system is disabled in the event of a malfunction.

The pressure sensor **1007** of FIG. **6** monitors the status of the energy storage device **1002** and compares the magnitude of the stored energy to a target magnitude. When the magnitude of the energy stored reaches the target value, the storage of energy is terminated. The target value may be a default value established by the manufacturer or a value established by the operator on the basis of experience with different subjects, e.g., adults, children, animals, and/or types of serum. DNA technology, agricultural procedures and different types of senim may be better served with different pressures. The target pressure value is one of the "initial conditions" which an operator may set by controls in the IC Set function **1010** of FIG. **6**.

The volume sensor **1008** provides assurance that a correct amount of liquid is used in each injection.

The velocity sensor **1009** of FIG. **6** determines the time required for the stored energy to decay to some predetermined value after an injection. The decay time is a measure of output port performance. If the output port is partially clogged, the pressure decays too slowly; and if the output port is worn or too large, the pressure decays too rapidly. If a failure is detected, a warning will be issued to the operator and the system is disabled until corrective action is taken.

The IC Set **1010** of FIG. **6** permits an operator to select initial condition values for the pressure sensor **1007**, the volume sensor **1008** and the velocity sensor **1009**.

The processor and decision logic **1011** issues control signals to the system power control **1013** and status signals to the monitor display and warning unit **1012**.

In addition to the control and monitoring function described above herein, the circuit arrangements of FIG. **4** maintain a record of the number of injections completed or, in the case of the cartridge system described below in relation of FIGS. **7**A and **7**B, the number of cartridges remaining as the cartridges in the magazine are used up.

The implementation of the system functions by the arrangements of FIGS. **3** and **4** will be understood from the following description.

Digital inverters **310** and **312**, resistors **314** and **316**, and capacitor **318** are configured to form a reference frequency oscillator. The operating frequency F_{r1} is determined by the time constant of the resistor **314** and the capacitor **318**. This oscillator, or a separate oscillator, can provide the pain suppression signal for electrical pain suppression function **319** and surface contact pads or guard ring **321**.

Digital inverters **300** and **302**, capacitor **308**, and variable sensing inductance **304** in FIG. **3** form a variable frequency reluctance transducer oscillator which has an operating frequency F_P. The operating frequency of the oscillator varies as a function of the value of the inductance in coil **304**. The coil **304**, which is not shown in FIG. **2**A, is mounted at the center of recess **107** and inside the energy storage spring **227** and is partially covered by the reluctance shield **226** of FIG. **2**A. It is noted that reluctance shield **226** is shown to the outside of spring **227** for illustrative clarity, however, it is ideally situated at the inside diameter of spring **227**, which is quite large in order to develop 1700 psi or more as needed for an effective deep penetration injection for human use or 6000 psi or more and for sufficient penetration of a dairy cow. In any event, a change in the relative position of the coil **304** and the shield **226** as the spring **227** is compressed changes the inductance of the coil **304**. Accordingly tile frequency of the oscillator, which is determined by the time constant of the inductance of coil **304** and the capacitor **308**, is determined by the degree of compression of the spring **227**. A reluctance transducer oscillator with a sensing inductance as described above is known from my U.S. patent application Ser. No. 07/625,942, filed Dec. 11, 1990, for "Inductance Systems." It is noted that other types of oscillator networks can also be used for these functions, for example, analog comparators or amplifiers.

Flip flop **320** is configured as a frequency mixer which provides a digital output signal which has a pulse rate F_{Z1} which is the difference between the reference pulse rate F_{r1} and the oscillator frequency F_P. In the absence of pressure on the spring **227**, the frequencies F_{r1} and F_P are equal and the pulse rate F_{Z1} at the "1" output of flip flop **320** is zero.

In the illustrative embodiment of FIG. **3**, energy enhancement techniques drive motor **221** with a series of high speed, high energy and relatively high voltage pulses. The output of

AND gate **338** controls the generation of the motor drive pulses. The inputs to the AND gate **338** comprise: the "0" output of the flip flop **336** which remains high until the target value of spring compression is reached; the BT conductor from pulse decoder **424**; the "0" output of the flip flop **380**, which is high except when the trigger is activated to initiate an injection; the output of the inverter **356**, which is high until the charge on capacitor **348** reaches a critical value; and the output conductor of the reference oscillator. When enabled, the output signal of AND gate **338** turns the FET **340** on and off at the rate F_{r1} of the reference oscillator. When the transistor **340** is on, current will flow from positive potential through inductance **342** and the transistor **340** to ground. When the transistor **340** is subsequently turned off, the energy stored in the magnetic field of coil **342** will discharge through the path which is comprised of diode **344** and capacitor **348**. The resistors **350** and **352** are of relatively high value; therefore, very little energy is lost in the path to ground through those two resistors. The magnitude of tile voltage generated by the collapse of the magnetic field of coil **342** is very high and is dictated by the rate of collapse of the field. The rate of collapse is determined by the impedance of the discharge path. The diode **344** prevents reverse flow of current due to the buildup of voltage on the capacitor **348**. Capacitor **346** is a stabilizing capacitor which provides an extra measure of current for the coil **342** during the ON state of transistor **340**. When the charge and corresponding voltage on capacitor **348** reaches a predetermined value the output of the threshold detector **356** will go low and gate **338** is disabled. The predetermined value represents a charge and voltage large enough to advance the motor **221**. When the output of detector **356** goes low, the output of inverter **358** goes high to enable transistor **360** to provide a path for discharging the capacitor **348** through the winding of motor **221**. As the charge is depleted and the voltage on the capacitor falls below the threshold value of detector **356**, the output of detector **356** goes high to enable gate **340** to initiate another cycle of charging capacitor **348**; and the output of inverter **358** goes low to disable transistor **360**. High speed charging cycles will continue until the flip flop **336** is set to the "1" state which indicates that the energy stored in spring **227** has reached the target value. In the drawing, the output labeled Q_S is the "1" output of the flip flop **336** and the complement output is termed the "0" output herein.

The energy enhancement technique of the FIG. **3** embodiment allows for a power source that has neither the voltage or current capability for directly driving the motor. However, if the power source has a voltage level that satisfies that of the motor, but whose electrical current capability is insufficient, then the drive embodiment of FIG. **3**A can be used. In this case, energy enhancement still applies, but the voltage amplification provided by coil **342** and diode **344** are eliminated and the corresponding loss in efficiency is eliminated as well. Instead, when all conditions for an injection are satisfied, gate **338** activates transistor **340** so that capacitor **348** is charged to the voltage level of the battery; that is, the mid-point of divider **350**/**352** is adjusted so that Schmitt trigger logic inverter **356** will change state when battery voltage is achieved on capacitor **348**, and immediately thereafter, the motor is driven with closure of transistor **360** as described for the FIG. **3** embodiment. This scenario provides a high speed, high energy, albeit lower voltage, sequence of drive pulses to the motor. Capacitor **348** is selected to provide sufficient electrical current for a long enough time to exceed the design value for motor advancement.

FIG. **3**B shows an embodiment whose power source has enough instantaneous energy to drive the motor directly, i.e., one with enough current and voltage capability for driving the motor with no energy enhancement at all. In this case, transistor **340**, coil **342**, diode **344**, capacitors **346** and **348**, divider **350**/**352** and Schmitt inverters **356** and **358** are all eliminated. With this embodiment, the output of gate **338** will now drive transistor **360** so that the motor is connected directly to the power source when all conditions for an injection are met. A prototype of the "direct drive" system with a 9 volt alkaline battery achieved more than 400 injections in excess of 1700 psi each. With this arrangement, load time per injection is only 10 seconds; however, load time can be increased or decreased with variations in motor size, gear ratio, power capability and/or target pressure. It is also noted that for the same system, over 1,000 injections are achieved when using a higher energy density lithium battery.

While all of the jet systems described herein are smaller, lighter in weight and more convenient to use than those of the past, the selection of one drive technique as opposed to another is dictated by economics, acceptable load time and the primary use for the injector. For example, the FIG. **3** approach is the most compact because the total energy enhancement described will allow for a smaller motor/battery combination, however, it is also the least efficient and will result in fewer "shots" for a given amount of initial energy. A good example for this system is its daily use by diabetics where load time is not so critical and where it is easily carried in a woman's purse or a gentleman's pocket. When the maximum number of injections is paramount, such as mass immunization by the military, veterinary, DNA or agricultural procedures, then a better choice is the other end of the spectrum as shown in FIG. **3**B, that is, using a larger, direct drive motor, with lower voltage, higher efficiency and faster load time.

Finally, because of the wide adaptability of the inventive system, some applications might use motors with very high start-up current and/or free-wheeling rotors. If this is the case, the motor is ideally driven with an optional, electrically driven, clutch or gear engagement. In this way, the inertia achieved by the rotor at start-up is preserved by disengaging when capacitor **348** is charging, re-engaged when the next pulse of energy is given to the motor and will again disengage when completed. The entire sequence is conveniently controlled with the same signal that turns transistor **360** ON and OFF as capacitor **348** charges and discharges in the enhancement techniques of FIGS. **3** and **3**A. When this is done, the signal to transistor **360** is synchronously delayed so that the mechanical linkage is optimally engaged at the same time that the energy stored in capacitor **348** is released into the motor. The feature can also be used with FIG. **3**B, but its advantage is not as great because, in this case, once started, the motor stays on until target pressure is achieved.

Regardless of the method used for driving motor **221**, flip flop **336** is controlled by the A_N output conductor of counter **322**, by an output signal of the digital window decoder **386**, and by the BT conductor. The flip flop **336** is set to the "1" state when the A_N output of the counter **322** goes high if the BT conductor is high; and is reset by the output conductor of the decoder **386**. Counter **322**, in turn, is controlled by the F_{d1} signal at the output of flip flop **320** and by the output of flip flop **362**. Flip flop **362** is set by a F_{d1} signal at the output of flip flop **320** and reset by a B_n output signal of counter **364**. Counter **364** defines a period of time in terms of pulses of the reference frequency F_{r1} and counter **322** counts the difference frequency pulses F_{d1}. Since counter **364** and

counter **322** are reset at the same time by an output signal of flip flop **362**, counter **364** provides a measurement window of time which runs from reset time to the next reset time. The A_N output conductor will remain low until the deformation of the spring **227** reaches the target value. When the counter **322** reaches the A_N count within the measurement time window, flip flop **336** is set and gate **338** is disabled. At the same time, the "1" output of flip **336** is transmitted to the warning function **388** to indicate that the device is ready for an injection procedure. Flip flop **336** can be set only if the "BT" input to the "D" terminal of that flip flop is high. As will be explained with respect to FIG. **4**, the BT conductor will be high if the bellows test is satisfactory. The digital code which is stored in counter **322** during a measurement time interval corresponds to the instant deformation of the energy storage spring **227**. The digital pulse decoder **324**, in response to the digital code in counter **322**, generates input signals for the BCD counters **326**, **328**. For example, if the deformation of the spring which is equivalent to one pound of force on the spring provides ten cycles of differential frequency F_{d1}, decoder **324** will convert the code in counter **322** to a single pulse for BCD counters **326**, **328**. With a count of one in the counters **326**, **328**, the BCD decoders **330**, **332** provide signals to the display **334** to display the value, one pound. Any number can be displayed with appropriate decoding by pulse decoder **324**. By virtue of the display **334**, the operator knows that the appropriate level of energy is stored in the spring **227** and that an injection may be initiated. The flip flop **336** remains set until an injection has been successfully completed. If the velocity test fails, a warning in **388** will issue and flip flop **336** will not be reset. Accordingly, remedial action must be taken before preparation for another injection can be started.

The power on switch **375**, in the lower left portion of FIG. **3**, connects positive potential to the input of inverter **379** through the contact segments **370** and **371**, detents **232** and **233**, and line **377**. The contact segments **370** and **371** lie in the recesses **108** on the back plate **104** shown in FIGS. **1** and **2**. When the trigger is operated, the detents **232** and **233** are disconnected from the contact segments **370** and **371**; and, because the input is referenced to ground through resistor **374**, the output of inverter **379** goes high. A high signal from the output of inverter **379** increments a counter in **390** to display the number of injections completed from the current bellows; and causes the "D"-type flip flop **380** to be set to the "1" state. Consequently, the "0" output of flip flop **380** goes low which disables AND gate **338**. The high signal on the "1" output of flip **380** enables AND gate **382** to pass F_{d1} difference frequency signals to the input of counter **384**. As explained earlier herein, the difference frequency will be reduced accordingly as spring **227** comes to some predetermined value after an injection. The count which is accumulated in the counter **384** is thus representative of the time required for the injection chamber to be partially collapsed. The window decoder **386** evaluates the count in the counter **384** on the basis of the expected values established by IC **2**. If the count is larger than the expected limits, it is probable that the output port is plugged, and if the count is smaller than the expected limits, it is probable that the output port is enlarged beyond acceptable limits. In either event, a warning signal is displayed by the warning indicator **388** and the flip flop **336** will not be reset until remedial action is taken. If the count in counter **384** is within limits, an output signal of digital decoder **386** will reset flip flop **336** and the BCD counters **326** and **328**. When that occurs, the cycle to drive the motor to load energy into the spring **227** will begin again. The time required for the chamber to partially collapse is short compared to the time required for the detents **232** and **233** to again settle in the recesses **108** and reconnect positive potential to the input of inverter **379**. This time relationship is positively assured if a dashpot is employed to slow the return as suggested earlier herein. When the positive potential reappears at the input of inverter **379**, capacitor **376** and resistor **378**, which are configured as a high-pass filter, produce a reset pulse to flip flop **380** and counter **384** in preparation for the next injection. In the event that a very large volume injection is to be performed, the time required to inject the fluid may exceed the time for the detents **232** and **233** to settle in recess **108**. In that case the illustrative high-pass reset circuitry can be replaced with circuitry with appropriate delay.

FIG. **4** provides an arrangement for testing the integrity of the liquid-filled bellows **404** or any other type of fluid-filled cartridge. Inverters **400** and **402** are configured as an oscillator in which the output frequency F_b is determined by the impedance across the entire bellows **404**. Inverters **410** and **412** are configured as a fixed frequency oscillator having a frequency F_{r2}; and flip flop **420** is connected as a frequency mixer for the signals F_{r2} and F_b. In the configuration of FIG. **4**, the collapsing bellows behaves as a variable resistance; therefore, the frequency of the mixer output signal F_{d2} is minimum when the bellows is full. As the bellows collapses, the impedance decreases and the differential signal F_{d2} increases. The counter **422** accumulates the Fd, signals during a measurement time interval defined by the "0" output conductor of flip flop **362** of FIG. **3**, and pulse decoder and display **424** displays bellows status information. The use of the time period provided by the D_N count is for purposes of illustration. In the event that a different time period is desired, additional counter outputs and flip flops are provided. The arrangements of **424** evaluate the interval count in counter **422** on the basis of the IC set 3 information which defines a range or window of acceptable values. If the count falls within the range of acceptable values, a high BT signal will be generated and flip flop is set on occurrence of the next succeeding A_N signal from counter **322**. However, if the serum within the bellows has excessive voids, clots or an incorrect consistency for some other reason, the F_b frequency will fall outside the acceptable range and the count in **422** will fall outside the preselected window of performance.

It should be noted that fluid may be used as a dielectric material in an alternative embodiment in which a variable capacitance determines the frequency F_b. In that embodiment the variable bellows is located at the position of the capacitor **408** and a fixed resistor placed at the position **404** in FIG. **4**. In this case, the two ends of the bellows form the capacitor plates and the serum fluid is the dielectric material. As the length of the bellows decreases, the capacitance increases and the frequency F_b decreases.

FIGS. **5**A to **5**D show a needle-type hypodermic injector in accordance with my invention in various stages in the use of the injector. The injector of FIG. **5**A comprises a bellows **500** sealed with end cap and ram **510**; a front housing **503**; a rear housing **502**; a pressure piston **501**; a needle output port **508** with a flange **511**; a bellows-shaped needle sheath **504**; and a removable cap **507**. FIG. **5**A illustrates a fresh injector prior to use. As in the injector embodiment of FIGS. **1** and **2**, the support guide rings **116** of those figures may be employed in the embodiment of FIGS. **5**A to **5**D. The bellows **500** may contain a liquid serum or a lyophilized (freeze dried) vaccine. In the latter case, a liquid which is stored in the sheath bellows **504** is driven into the bellows **500** as described below herein. The bellows **500**, the rear

housing **502** and the front housing **503** all may be fabricated of clear plastic material so that the operator can observe whether or not blood is drawn into the bellows **500** when the pressure piston is slightly withdrawn.

The breakaway seal **512** and the cap **507** are removed to permit the operator to depress housing **502**. The need to remove the cap **507** may be eliminated if the cap **507** is made of a self-sealing material, e.g., pure latex rubber. Typically, the thumb is placed on the pressure piston **501** and index finger and the adjacent finger are placed on the flange **511**. The resistance of the sheath bellows **504** is sufficient to cause the bellows to expand after use; however, the resistance of the bellows **504** is small compared to the force required to compress the liquid bellows **500** to eject the liquid through the needle output port. Therefore, as pressure is applied between the flange **511** and the pressure piston **501**, the sheath bellows will begin to collapse and needle exposure will begin. As the sheath bellows is collapsed, the right-hand side **520** of needle **506** will puncture the membrane separating sheath bellows **504** and serum bellows **500**. As compression continues, the liquid residing in sheath bellows **504** is forced into serum bellows **500** to form the fluid state of the desired serum. Ultimately, needle flange **505** will engage the surface **514** therein, after removal of breakaway seal **513**. Continued pressure will force the serum in **500** to be expelled through the exit port of needle **506**, said serum being unable to re-enter **504** because it has collapsed to zero internal volume. A foam ring **516** positioned on the right side of flange **505** serves as a cushion to prevent flange **505** from opening the membrane to a greater extent than that of puncture point **520**. The membrane in **500** also can be made of self-sealing latex diaphragm material which will tend to hold the needle in place after the injection is completed and the sheath is again extended to cover the needle. The first intermediate state of the injector is illustrated in FIG. **5**B.

In cases where the serum is stored in bellows **500** in a liquid state, the bellows **504** can be replaced with a simple coil spring. However, if as suggested earlier herein, the vaccine is stored in the lyophilized state, the fluid required to turn the vaccine to the liquid state is stored in the bellows **504**. In this latter case, the liquid in bellows **504** is forced into bellows **500** through a hole in the membrane of the bellows **500** which is breached when the bellows **504** is first compressed to begin exposure of needle **506**.

After the needle is inserted into the injection site, the breakaway seal **513** is removed and pressure is applied between the pressure piston **501** and the flange **511** to collapse the liquid bellows **500** and eject fluid through the output ports into the injection site. The state of the injector after depletion of the injection fluid is illustrated in FIG. **5**C. As shown in FIG. **5**E, a sawtooth pattern **518** on the outer surface of the pressure piston **501** and a single sawtooth **517** on the inner surface of the rear housing **502** permit the pressure piston **501** to be advanced into the member **502** and thus compress the bellows **500**. However, the cooperation of **517** and **518** prohibits withdrawal of the piston **501** after engagement of **517** and **518**. As an option, the end of the ram **510** is shaped to strike and crush the end **520** of the needle **506** when the bellows **500** is fully collapsed. This will further assure that the needle injector cannot be reused and will tend to retain the needle in engagement with the bellows **500** when the sheath bellows **504** and the sheath **503** are extended to cover the needle.

After the needle is removed from tile subject and pressure between **501** and **511** is removed, the bellows **504** expands as shown in FIG. **5**D. As is seen in FIG. **5**D, when the needle sheath bellows **504** extends to its full length, the needle **506** is withdrawn from the needle guide **508**. This occurs because, in preparation for the injection, end **520** of needle **506** penetrated the membrane to breach the serum chamber. Because the needle guide opening **508** is small compared to the trap opening **509**, it is difficult if not impossible to again collapse the bellows **504** without the end of the needle **506** hitting the end wall **515** of the trap section **509**. The tendency of the needle to hit the wall **515** can be enhanced by imparting a small bend in the needle **506** prior to initial installation into the guide opening **508**.

The above discussion for FIGS. **5**A to **5**D deals with the various steps for a special case insertion of a hypodermic needle into an injection site. Capsules **5**A to **5**D and needle **506** can also provide several other valuable functions. First, if the housing is designed to fit into a jet injection chamber similar in nature to that of FIG. **7**, needle **506** issues the highly desirable dispersion pattern of a jet injection stream much deeper into the body. This capability provides superior medical treatment in some cases. Second, if needle **506** is shortened to exit the capsule to the appropriate length, it will deliver lyophilized medications while deriving all of the advantages of a perforator jet injection. Third, if needle **506** is made even shorter by the correct amount, it will not exit the housing at all, but instead, the capsules **5**A to **5**D will serve as a non-penetrating, flat, prior art orifice, similar to that shown in FIG. **9**D but capable of delivering a lyophilized jet injection to humans or thinner-skinned animals.

FIG. **7** shows an alternative embodiment of a jet injector system in which the serum for each and every injection is individually contained in its own collapsible cartridge. A dispenser **700** includes a handle **911** from the forward end of which extends a trigger **701**. A muzzle **913** includes at its rearward section a lever **702** extending laterally through a longitudinal slot **915** in muzzle **913** which terminates at its forward end in a downward extending portion **917**. Dispenser **700** includes at its forward end a receptacle or opening **919** for receiving a cartridge **800** holding a serum for injection into a recipient. Dispenser **700** includes a piston or ram **703** for acting on cartridge **800** to effect an injection. Cartridge **800** is described below and shown in FIGS. **8**A and **8**B.

For illustrative purposes, tile cartridge **800** shown in FIG. **7** is grossly oversized, wherein a normal injection requires from ½ to 1 cc of serum (from about 0.031 to 0.061 in^3) and the actual cartridge size is commensurate with that volume. Since the system of FIG. **7** can be proportioned to handle single-shot or multiple-shot cartridges, the smaller size is also true for the magazine embodiment of FIGS. **7**A and **7**B, that is, the illustrative magazine is many times larger than that needed for the N cartridges shown in the figures. For a single-shot arrangement, the lever **702** is drawn to the rear to permit removal of a used cartridge from receptacle **919** and a fresh cartridge installed. After a fresh cartridge is installed, the lever **702** is moved forward, to the left in FIG. **7**.

With regard to the magazine embodiment of FIGS. **7**A and **7**B in which the N cartridges **800** are serially introduced into system **700**, which is shown identical to that in FIG. **7**, many magazine configurations are possible for sequential loading into and out of the injection chamber. For example, much in the manner of a modern pistol with a linear magazine containing N bullets or, the revolver-type weapon in which the bullets rotate into the chamber or, as shown in the oversized preferred embodiment of FIGS. **7**A and **7**B, there is shown the entire magazine **921** which is simply detented through opening **919** in the injector chamber as each new

injection occurs. In this latter arrangement, the cartridges are not extracted at the completion of each injection, but simply remain in magazine **921**. This feature is especially important for the prevention of unsafe littering at the scene of mass immunization programs such as the military or activities of the World Health Organization in their worldwide efforts with disease control. Other methods of collection and storing the used cartridges are also possible, for example, a magazine that has an evacuated volume equal to that of the loaded cartridges, and into which the used cartridges will drop at the conclusion of an injection.

A manual advance for the magazine can be implemented with lever **702** or an automatic advance is realized with a spring configured ratchet assembly that is "wound up" when magazine **921** is slipped into the bottom opening of the dispenser **700** and urged upward until latched into the initial position for the injection sequence. Since the system is electrical in nature, a motor advance is the most convenient of all; however, power conservation for a multitude of high pressure injections has a higher priority in many applications. As discussed earlier, any suitable driving force can be utilized to drive pressure piston **703** forward to collapse the serum chamber **804** in cartridge **800** (as explained below) when said cartridge appears at the injection site.

Switch **375** is basically the ON-OFF switch for system power. The manner in which the power is provided can be carried out in a number of ways, wherein the one or more ways selected is normally dictated by the application and/or user preference. Several embodiments are described below.

Referring to FIG. **3** for one such way, switch **375** is simply turned ON, after which flip flop **336** automatically enables gate **338** and turns ON motor **221**; when target pressure is detected, flip flop **336** disables gate **338**. This cycle continues until a system failure occurs or the injection capability is depleted. In a single or multiple cartridge system, the detent action of contacts **232**, **233** is provided as part of the trigger mechanism **701** in FIGS. **7**, **7**A and **7**B.

Turning to FIGS. **7**A and **7**B for another embodiment, momentary switch **372** in FIG. **3**C replaces the latching contacts **232**, **233**. Switch **372** could be temperature sensitive and would only be enabled if a temperature threshold is reached. Moreover, switch **372** also can only be enabled if an access code is entered, as for example by an access key. Switch **375** still provides primary power to the system, but switch location **372** will remain open until all of the following requirements are met: (1) the magazine is securely positioned in the dispenser, after which switch **372** closes as it engages the first cartridge through slot **373** in the wall of magazine **921**, (2) switch location **372** is enabled when the user applies pressure to guard ring **103** or **805** when the ring is brought into firm contact with the injection site, (3) the temperature threshold is met, and (4) the access code is entered. These features provide additional levels of safety by preventing accidental firing of the injector until the user is ready and/or prevents use by illicit drug users or other unauthorized people. In the case of temperature, damage is prevented to the injector or user when the injectant, whose viscosity is temperature-dependent, is too thick to allow for a safe and timely passage through the small diameter orifice under the influence of very high pressure. Other embodiments of switch location **372** are also possible and will depend on the level of safety required for a particular application.

A MANUAL/AUTO select is provided in another embodiment, wherein the manual mode switch location **372** is actuated by the user rather than by the cartridge, the guard ring or temperature detector in the AUTO mode. However, the AUTO mode is also provided, as described in the preceding paragraph.

When the magazine and cartridge embodiment is used, counter and display function **390** will electronically measure and display the number (shown as **926** in FIGS. **7**A and **7**B) of "live" cartridges actually found in the magazine. The counter and display number will then proceed with a count-down to zero as the cartridges are used. Again, this feature is important for situations where a magazine is removed and then returned to the dispenser for a later round of injections.

It is clear that in the magazine-type system, the expended cartridges can be safely disposed of; however, for purposes of economy in certain situations, the magazine can be ruggedly built for multiple long term use, in which case the entire magazine can be returned to a center for disease control, sterilized and reused in whole or in part. In one such embodiment, the cartridges themselves are reusable, but the exit nozzle is removed and replaced in the same manner as described for the multiple-dose cartridge of FIGS. **1** and **2**. If the magazine is reusable, a preferred embodiment of this feature has the cartridge counter, its processor, display and a power source ideally located right in the magazine. With this embodiment, no count initialization is necessary because it simply detects the live cartridges found in the magazine on a real time basis whether the magazine is in the dispenser or not. In fact, this technique is useful for other magazine-oriented applications such as pistols, shotguns, rifles and automatic or semiautomatic weapons of any type. For such a system, a low battery warning would be used with the independent counter system as well. A specially designed version of the reluctance transducer and processor described for FIG. **3** is ideally suited for this embodiment.

Finally, as pointed out earlier, it is noted that a dispenser similar to that of FIG. **7** can be used for the needle oriented cartridge of FIGS. **5**A–**5**E. Further, if the FIGS. **5**A–**5**E cartridge is designed into the magazine structure of the FIG. **7**A dispenser, then N needle-type cartridges can be administered in much the same way as the jet injector embodiment, but with greater efficiency and speed than that of the individual cartridges, while still providing the highly desirable dispersion pattern of a jet stream, and with even less risk to the population because the entire magazine remains under the watchful eye of the immunization team. As in the case of the FIG. **5** embodiment, the jet injector of FIGS. **7**, **7**A, **7**B, **7**C, **7**CC, **7**D, **7**E, **8**A and **8**B can be fabricated of clear plastic material so that the operator can observe if the needle has penetrated a blood vessel after insertion, but prior to expelling the fluid. This is possible by virtue of an automatic micro-withdrawal of the pressure piston under the influence of the monitoring and control sequence of the electrically driven system.

FIGS. **7**C, **7**CC and **7**D offer alternatives to FIGS. **7**A and **7**B by allowing for a rechargeable power source and an even larger multi-shot system. Typical applications are veterinary and agricultural uses where the multiple-shot cartridge system is replaced by serum container **962**, or for use at remote locations where grid power is not available.

Viewing FIG. **7**C, the correct dose of serum **960** is pulled out of container **962** through connecting tube **965** and into the injection head through access nipple **966**. This will occur each time the ram is pulled back in preparation for the next shot, wherein every dose is administered through the same orifice **969**. For veterinary use in particular, the periodic (sometimes daily) administration of the BST hormone to a herd of dairy cows becomes a very efficient procedure. With

this scenario, serum container **962** is pre-loaded with the correct number of doses and is then carried by the user on waist or shoulder harness **970** as shown in FIG. 7CC, and the entire herd can be quickly inoculated. Also shown in FIG. 7CC is an additional fluid container **963** with a dye material that is transported to exit nozzle **967** through connecting tube **964**. Especially useful for the injection of large animals in close proximity to each other, an appropriate amount of dye is drawn into the dye chamber at the same time serum is drawn into its chamber. The dye is expelled and deposited at the injection site as an indicator to show which of the animals has been injected with a particular material. This feature will prevent the adverse effects no injection at all or having an animal receive the same material more than once.

In FIG. 7C, power pack **950** is inserted and removed from access port **954** of the jet injector housing, wherein electrical contact **952** connects power to the injector. With this approach, the number of shots available from each source is not quite so critical, whereas it would be inconvenient for members of an immunization team, health care, agricultural or veterinary workers to repeatedly change and dispose of the battery. All they have to do is carry a number of sources in a storage harness, change them as each is depleted, and then have the entire group recharged with solar energy for isolated areas or a standard AC or DC source at more convenient locations.

Finally, power adapter **968** in FIG. 7D can be inserted into the injector housing in place of power pack **950** to allow for operation from other power sources if the portability of small battery operation is not required. For example, adapter **968** can be connected directly to an adequate source of external power or to power pack **972**, wherein the injector is then powered with a non-disposable source of external energy that is transported to the remote location along with the injector, the medications and the health worker units. External source **972** can be a high capacity rechargeable battery or a storage capacitor whose value is large enough to supply N shots before recharge is needed. Recharge of power pack **972** can come from grid power at socket **976** (if available), or as an alternative, a solar panel trickle charger **978**, a portable generator **980** or directly from a vehicle's power system **982** if such vehicle is available to the health care workers. In some remote locations, powerful solar systems **983** located nearby or on the roof of the health care facility are also conveniently used.

An injector system **703** of FIG. 7E illustrates a form-fitted, barrel-like restraining chamber **705** defined by a cylinderical barrel **707** forming part of the barrel and mounted on a hinge **709** and a cooperating door **708** which together provide the support needed for a high pressure injection with an inexpensive, thin-walled capsule **820**. Capsule **820** can have a wall thickness of from 0.01 inch to 0.05 inch. These capsules **820** are discarded after each injection in the same manner as the disposable needle and syringe, except that the inconvenience, danger and cost of disposal for needle injections are eliminated. To load a capsule, door **708** is opened and capsule **820** inserted into restraining chamber **705**. An access groove **710** accommodates and supports the guard ring end **103** of capsule **820**. This end of capsule **820** also has a non-slip surface **901** as well as exit orifice **109**. After capsule **820** is inserted, door **708** is secured in the closed position with a latch **712**. The form-fitting chamber **705**, a lip **714** on door **708** and groove **710** work together to hold capsule **820** securely in place to prevent any damage during the high pressure injection. A spring loaded arm **716** having a spring **717** attached to the interior of barrel **707** is compressed when the capsule is inserted and ejects the capsule when door **708** is opened; thus, physical contact and the risk of cross-infection for the health worker is further reduced when the injection is completed. Other parts of the injection system similar to those of the apparatus of FIG. 7D are given the same reference numerals.

The FIG. 7F embodiment has good utility with large scale injection programs for animals and is an alternative to tile replaceable capsule. Subsystem **830** is a low cost combination comprising serum sack or chamber **832**, connecting passageway or tube **834** and thin-walled injection capsule **836** all supplied as a single unit. When a particular medication is given to a herd of cattle, serum sack **832** is filled with the selected medication and inexpensive capsule **836** is inserted into the highly restrained injection chamber **705** of injection system **703**. When the injection procedure is completed, the entire subsystem **830** is removed and stored for future use or discarded. This system will avoid the problem of gumming-tip the injector for situations and/or locations that make it inconvenient to clean the device in a timely manner as, for example, for large numbers of feed cattle on the range or when they just come in for treatment.

FIGS. 7G and 7GG show a capsule or injection barrel **840** intended to illustrate two different embodiments of an injection head with multiple exit ports. In the first case shown in FIG. 7G, a set of multiple exit ports **842** provide a number N jet stream of a fluid **843** from a single serum chamber. This technique has utility for advantageously reducing injection time for very large doses, sometimes in excess of 5 cc for large animals such as cows. With small exit ports on the order of 0.004 to 0.012 inch as required for a high velocity, piercing jet stream, the amount of time needed to discharge the fluid with a single port is quite long, up to three seconds, giving the animal ample opportunity to move before the injection is completed. This first embodiment is intended to avoid that problem.

The second embodiment is shown in cut-away form in FIG. 7GG and is useful where discharge time is not a factor, for example for smaller human doses in the order of 0.5 to 1.0 cc. However, for this case, the multiple exit port configuration has even greater utility for injecting more than one vaccine into the injection site at the same time. This is especially important for children where repeated use of needle injections is frightening, painful and very often difficult to complete because of adverse physical resistance, as well as the added expense of return visits to the health center or doctor's office. This type of patient reaction could lead to inadequate vaccinations and the possibility of unnecessary exposure to some very dangerous diseases. The multiple exit port solution makes use of a number N parallel, independent, barrel-type restraining chambers, one of which is shown as **845** in the cut-away view of barrel **840**. Included in each of restraining chambers **845** is a different, inexpensive, thin-walled vaccine capsule and its exit port **842'** that rests against a mating hole **838** in a forward access disk **844**, shown as a broken out structure in FIG. 7GG. These capsules are similar to that described for FIG. 7E, but thinner in diameter to facilitate the side-by-side orientation. Each of the N capsules has its own piston **847** which interfaces with N geometrically matching rams **848** appended to a single central ram **849**. Ram **849**, and each of rams **848** moves to the left as shown in the drawing, pushing pistons **847** inward with the correct force when released from its position of stored mechanical energy. This configuration allows the health worker to rapidly dispense all injectants at the same time so that pain, fear, expense and resistance to injections are all dramatically reduced. In the

description of FIGS. 7A and 7B, it was noted that a multiple chamber system similar to a revolver type pistol could be used for housing the vaccine capsules. The capsules can be loaded in the configuration of FIG. 7GG by sliding barrel **840** forward to expose the N mating carriages into which capsules **846** are inserted. The form-fitted outer portion of barrel **840** is then slid back into position to securely lock capsules **846** in place until the injection is completed. A trap door similar to door **708** described for FIG. 7E can also be used. Capsules **846** can be prefilled by the vaccine manufacturer and shipped to the health center, or they can be filled by the health worker with the appropriate vaccine when the person shows up for the injection. If jet or needle capsules of the type described for FIGS. 5A–5E are used, lyophilized serum can also be administered simultaneously with either the needle or jet orifice embodiment.

The multiple capsule system of FIG. 7GG and 7GG' has further utility by providing for different injection depth from each of the N injection chambers. This is accomplished by simply making a small change in the cross-sectional area of each of the capsules and its piston **847**. Since central ram **849** will apply the same force to each of the independent rams **848**, chamber pressure will depend on the cross-sectional area of the piston to which the force is applied. If this is done, slight changes in capsule does will occur since the distance of travel for all rams is the same because of their dependence on central ram **848**. By the same token, the risk of error could be reduced because the containment chambers **845** can also have cross sections that will only accommodate a predetermined capsule. However, if consistent dose is an issue, further flexibility for injection depth is realized with slight modification to the diameter of the jet orifice for each capsule to facilitate a change of stream velocity for the known force, and injection depth will vary accordingly. Finally, different penetration depth for each capsule will help keep the injection products farther apart in the tissue, which in some cases is an important factor.

FIG. 7H illustrates a robotic system **850** for use with cattle **856** that are automatically identified and cut from the herd when an injection procedure is scheduled. The robotic system includes a control box **851** and an articulated, movable, mechanical arm **852** composed of injectant delivering tubes. This is a convenient time to mechanically restrain the animal, wherein arm **852** will move into position so a perforator **854** can contact the animal for a subsequent injection. The anchor point provided by the perforator is especially useful for a good injection because energetic movement by the animal is very likely at this time.

Further utility is provided if arm **852** of robotic system **850** is removable to provide a mobile, extended-arm injection system. A long, mobile injection system of this type will facilitate long range injections of large animals. This situation often arises if the animal is restrained at the neck, but requires an injection at a posterior portion of the body. The device is also useful when numerous animals are penned up, are in close proximity to each other, and are often lying down. For these situations, an extended arm on the order of one meter long will help simplify the injection procedure.

The cartridge **800** is shown in detail in FIGS. 8A and 8B. Cartridge **800** comprises a sealed serum bellows **804** with a ram **803**; a rear housing **801**; a pressure piston **802**; a front housing **812**, which comprises a guard ring **805** and a jet output port **807** with a flange **808**; and removable cap **810**. FIG. 8A illustrates a fresh injector installed in the system of FIGS. 7, 7A or 7B prior to breaching the seal of serum bellows **804**. As described elsewhere herein, cartridge **800** can be configured with a needle or perforator for delivery of the serum.

FIG. 8B illustrates the arrangements of FIG. 8A after the lever **702** of FIG. 7 is moved forward to breach the seal of the bellows **804** and to bring the injector piston **703** in position to drive the pressure piston **802** forward to collapse the bellows **804**.

FIGS. 9A–9D show the hide or dermis **860** of an animal such as a cow overlaying the subcutaneous space or layer **862**, which in turn lays over a muscle **864**. Dermis **860** has a thickness D and subcutaneous layer **862** has a thickness S. FIG. 9A illustrates the "pooling" effect that occurs when a capsule is used with a conventional needle and comprises an injection assembly **870** including a capsule **871**, a cylindrical housing **872** and a plunger **874**. A conventional hypodermic needle **876** is operatively connected to capsule **871**, which holds an injectant **878**. When plunger **874** is depressed, injectant **878** is ejected through the exit port of needle **876** and forms a pool **861** in muscle **864**. Pooling often provokes an attack by the animal's immune system, thereby causing an encapsulation of the medication which often leads to abscesses and/or serious scarring of the tissue. In the case of feed animals, damage to the meat causes a significant economical loss to the industry, and in some cases, will provoke a level of infection that renders the animal useless.

FIG. 9B illustrates an injection assembly **880** having capsule **871** with housing **872** and plunger **874**. A perforator **882** has a surgically sharp tip **883** at its delivery portion, and is attached to capsule **871** and enclosed with a collapsible protective housing **884**. Perforator **882** has an attachment portion **914** for attachment to capsule **871**. Collapsible housing **884** is in the form of a bellows having a free end **886** with walls **888** defining a circular opening **890** with a diameter larger than that of perforator **882**. Housing **884** has an opposite end **892** which is fixed to the base of capsule **871**.

Referring to FIG. 9BB, a detailed description of perforator **882** is shown. The perforator is of tubular construction, preferably made from stainless steel, having an effective length of no greater than the thickness of the dermis and subcutaneous layers of the skin or hide as discussed below. It has a slanted, surgically sharp tip **911** for being inserted into the skin or hide to provide a jet injection as explained hereinafter. Perforator **882** has a longitudinal, central axis L, and the slanted tip **911** has an oblong opening **913** with a central axis L' transverse to axis L. The slanted opening assists in preventing the delivery of foreign matter into a body during an injection with perforator **882**. The slanted tip creates an anchor point to establish and maintain the penetration position of the delivery portion of the needle. It further maintains laminar flow of the jet stream emanating from the perforator, even if there is movement of the body being injected.

In FIG. 9C, perforator **882** has been inserted into hide **860**, but its tip **883** falls just short of the subcutaneous layer **862**. Perforator **882** has an effective length X for insertion into the body having dermis **860**, subcutaneous layer **862** and muscle **864** for an intramuscular or subcutaneous injection. Length X is less than the sum of thicknesses D and S of dermis **860** and subcutaneous layer **862** so that it does not enter muscle **864** during an injection. Plunger **874** has been depressed and housing **884** has been collapsed against hide **860**, and tile injectant discharged to yield the penetrating jet stream **894** which will then deposit a vast majority of the injectant in the target area of the muscle **864**, if that is the desired target, while leaving virtually none in the hide **860**. If a subcutaneous injection is needed, one simply must reduce the injection pressure so that the jet stream will

penetrate the remainder of the hide while not having enough energy to successfully penetrate the muscle.

Finally, while this entire discussion has focused on perforator advantages for an intramuscular injection, both veterinary and human immunologists have cited experimental evidence indicating that injections into the dermis may be many times more potent than that of the IM or subcutaneous regions, so much so, in fact, that greatly reduced volumes of the injectant may be possible. If continued research in this regard leads to a recommendation that certain injections be given in the dermis, the perforator concept is remarkably effective for this application as well, and actually provides additional advantages over that of the needle and syringe. To assure a perforator intradermal injection, one must simply shorten the length X so that it penetrates the outer layer while at the same time falling short of the subcutaneous space. In addition, if the pressure is reduced to the appropriate level, experimental work by the inventor on a freshly euthanized cow has shown that all of the injectant will remain in the dermis, with none at the surface, while also spreading the injectant over a much wider radius than that experienced with the pooling effect of the prior art needle and syringe. As with the IM or subcutaneous injection, the increased area and volume covered by the injectant will provide an increasingly rapid and effective pick-up by the immune system.

The range of thicknesses of the skin, hide or dermis for humans and a variety of animals is known, and are set forth below:

humans: 1.0 mm–12.7 mm
cattle: 4 mm–8 mm
goats: 1 mm–5 mm
dogs: 1 mm (minimum thickness)
horses: 1 mm–5 mm
pigs: 2.7 mm–4.7 mm

Therefore, for dermal injections, the perforator should be less than 1 mm for any animal (or person) such as humans, goats, dogs and horses at their minimum skin thickness, less than 2.7 mm for animals such as pigs, at their minimum skin thickness, and less than 4 mm for animals such as cattle at their minimum skin thickness. Likewise, the perforator could be longer for injecting into humans or other animals at thicker parts of their skin but less than 12.7 mm (humans), 5 mm (animals such as goats and horses), 4.7 mm (animals such as pigs) and 8 mm (animals such as cattle).

FIG. **9**D illustrates the jet stream coming from a conventional prior art flat injection orifice. An injection assembly **896** includes capsule **871** with housing **872** and plunger **874**, and a flat jet orifice **897**. When plunger **874** is depressed, a jet stream **898** is emitted, and beginning from the outer surface of the hide **860**, the tough, hairy and often dirty conditions immediately often cause degradation of stream **898** so that a high percentage of the injectant remains on the outside, with very little, and in many cases, none of the injectant reaching subcutaneous space **862** or muscle **864**, resulting in a much less effective injection, even if a dermal injection was intended.

FIG. **9**E is an alternate embodiment of the invention showing perforator **882** from FIGS. **9**B and **9**C, but with a different orifice. In this case, a high quality, smaller diameter orifice **899** is located at the entrance end or port of perforator **882**. When the jet injection is initiated, it will travel down the middle of perforator **882** without touching the walls and before encountering any of the animals hide or flesh. The very high quality, high coherency of such a flow pattern **900** will allow for much deeper muscle penetration with even less of the injectant being left behind in the dermis **860** or subcutaneous layer **862** if the objective is an intramuscular injection.

FIG. **9**F shows injection system **880** with a high quality orifice **902** connected to the injection chamber of housing **872**, and having an externally threaded connector ring **904** affixed thereto. A perforator **906**, similar to perforator **882** but having an internally threaded entrance end or port **908** for engagement with the threads on connector ring **904**. The threaded connection between connector ring **904** and perforator **906** provides for an easy change of perforator **906** by simply unscrewing perforator **906**. Other fast connect-disconnect devices can be used as well. The system emits jet stream **900** as noted with respect to FIG. **9**E.

System **800** in FIG. **9**G is similar to that in FIG. **9**E, except that the high quality orifice **899** is located at the exit end of perforator **882**.

The injection systems according to the invention, such as shown for example in FIGS. **9**B, **9**E, **9**F and **9**G have generally flat front faces through which the perforator is extendable. The flat face helps prevent the hair and unsanitary material (such as that stuck on the hair) from being urged towards the injection site. The larger cross sectional area of contact around the injection site helps avoid the urging of tissue movement. The face need not be absolutely planar, but could have slight curves, a rough surface, indentations or the like, while preventing the urging of the foregoing movement.

Finally, FIG. **9**H illustrates an adaptation derived from the perforator concept. In this embodiment, small diameter exit tube **907** extends about 2 to 4 mm from injector housing **871** and is configured with a non-sharpened, flat ended output. This diagram is not shown to scale and is drawn primarily for illustrative purposes. With this embodiment, there is no initial perforation, but it was found that the short protrusion will move aside any hair and both stretch and provide a detent at an injection site **909** when surface contact is made. Significant improvements in jet penetration is realized, while at the same time reducing the amount of injectant left at the surface as opposed to that of the prior art flat orifice of FIG. **9**D. Benefits are found for human injections, for thinner hides of young calves and for other thin-skinned animal applications such as cats, dogs, fowl, etc.

The illustrative embodiments of our invention which are disclosed herein are but representative of our invention and many changes in form and function can be made without departing from the spirit and scope of our invention.

What is claimed is:

1. Apparatus for injecting fluid into a desired section of a body having an outer dermis and an inner region including at least a subcutaneous region and for some parts of the body, a muscular region, said apparatus comprising:

a fluid supplying device for supplying fluids at values of pressure and velocity of sufficient magnitude to generate a jet stream, and to inject a substantial amount of the fluid into a selected one of the outer dermis and the inner region; and

a perforator for making a perforation and entering the dermis of the body, said perforator comprised of an elongated tubular member having a first end connected to said fluid supplying device and an opposed sharp second end for perforating the body and dispensing the jet stream of fluid into the perforation, said perforator having an effective length of less than 12.7 mm, said effective length preventing said perforator from perforating the muscular region;

said fluid supplying device having a generally flat face through which said perforator is extendable.

2. Apparatus according to claim 1 wherein said perforator has a longitudinal axis extending from said first end up to said second end, and said second end has a central axis slanted relative to the longitudinal axis of said perforator for enabling said second end to penetrate the dermis and create an anchor point to establish and maintain the penetration position of said second end, and maintain an effective fluid flow of the jet stream even if there is movement of the body being injected.

3. Apparatus according to 2 wherein said second end has a surgically sharp end for piercing the dermis.

4. Apparatus according to claim 1, and further comprising:

orifice means positioned in said perforator for generating a coherent stream for flow through said exit portion.

5. Apparatus according to claim 1, wherein said perforator has an effective length of less than 4 mm.

6. Apparatus according to claim 1, wherein said perforator has an effective length of less than 1.5 mm.

7. Apparatus according to claim 1, wherein said perforator has an effective length of less than 9.5 mm.

8. Apparatus according to claim 1, wherein said perforator has an effective length of less than 3.1 mm.

9. Apparatus according to claim 1, wherein said perforator has an effective length of less than 8 mm.

10. Apparatus according to claim 1, wherein said perforator has an effective length of less than 5 mm.

11. Apparatus according to claim 1, wherein said perforator has an effective length of less than 1 mm.

12. Apparatus according to claim 1, wherein said perforator has an effective length of less than 4.7 mm.

13. Apparatus according to claim 1, wherein said perforator has an effective length of less than 2.7 mm.

14. Apparatus according to claim 1, wherein said perforator has an effective length of less than 4.2 mm.

15. Apparatus according to claim 1, wherein said perforator has an effective length of less than 3.6 mm.

16. Apparatus according to claim 1 wherein said perforator is removable and replaceable with another perforator.

17. Apparatus according to claim 1 and further including protective containment means for protectively containing said perforator before and after said perforator makes a perforation and enters the dermis of the body.

18. Apparatus for injecting fluid into a desired section of a body having a dermis and an inner region including at least a subcutaneous region, and for some parts of the body a muscular region, said apparatus comprising:

an electro-mechanical, spring energized fluid supplying device for supplying fluids at values of pressure and velocity of sufficient magnitude to generate a jet stream, and to inject a substantial amount of the fluid into a selected one of the outer dermis and the inner region; and

a perforator for making a perforation and entering the dermis of the body, said perforator comprised of an elongated tubular member having a first end connected to said fluid supplying device and an opposed sharp second end for perforating the body and dispensing the jet stream of fluid into the perforation, said perforator having an effective length of less than 12.7 mm, said effective length preventing said perforator from perforating the muscular region;

said fluid supplying device having a generally flat face through said perforator is extendable.

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Review

Advances in vaccine delivery: transcutaneous immunisation

Gregory M Glenn, Tanya Scharton-Kersten & Carl R Alving

Needle-free delivery of vaccines has become a global priority. Transcutaneous immunisation (TCI), topical application of vaccine antigens to the skin, can elicit systemic antibody and T-cell responses, suggesting that this new technique may provide a means for vaccination without needles. TCI requires the use of an adjuvant such as cholera toxin added to a vaccine antigen, such as diphtheria toxoid, to induce antibodies to diphtheria toxoid. The adjuvant and antigen are thought to target Langerhans cells, potent antigen-presenting cells found in the superficial layers of the skin. TCI appears to be a highly practical technique for delivery of vaccines that provides unique access to the immune system.

Keywords: *adjuvant, cholera toxin, heat-labile enterotoxin for Escherichia coli, Langerhans cells, transcutaneous immunisation*

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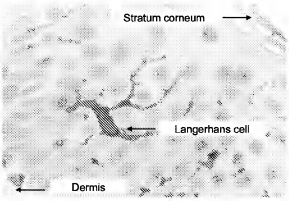
1. Introduction

Transcutaneous immunisation, the simple introduction of antigens to the host using a topical application to intact skin, is a new technique with both practical and immunological merits [1,2]. In practical terms, a needle-free method of vaccine delivery, such as TCI, will decrease the risk of needle-borne diseases, improve access to vaccination by eliminating the need for trained personnel and sterile equipment, and possibly provide a simple means for multivalent or multiple boosting immunisations. The immunological implications of TCI are potentially even more profound, as this technique appears to target highly accessible antigen presenting cells in the skin [3] that can be exploited for a variety of immune outcomes [4,5]. We have found that TCI can be reliably and reproducibly conducted with a variety of antigens to induce potent and functional immune responses [2,6,7]. Thus, this new method may significantly impact both the delivery of vaccines and open a new avenue for manipulation of the immune response.

2. The need for needle-free delivery of vaccines

Although vaccination represents the most effective medical intervention known [8], it continues to be under-utilised [9]. New methods of vaccine delivery have received much attention due to the need for multivalent or multi-boosting regimens and the need to reduce the barriers to immunisation and simplify procedures in the face of the burgeoning number of vaccines, both under development, and currently licensed [10]. While injectable vaccines are effective and widely used, they can be painful, and proper administration requires sterile technique, skill and trained personnel [11]. Needle-free delivery has become a global priority because of needle-born diseases associated with reuse and the improper disposal of needles

Figure 1: Human Langerhans cell (400 x magnification) in the epidermis. Although the majority of Langerhans cells are located in the suprabasal layers of keratinocytes, their dendritic processes appear within 1 or 2 cells of the last viable layer of keratinocytes.



[12]. The benefits of topical application of vaccines, delivered in the form of a patch, may provide greater access to vaccines, avoid the difficulties arising from the use of needles and assist in the implementation of multiple boosting and multivalent vaccine regimens.

3. The theoretical basis for TCI

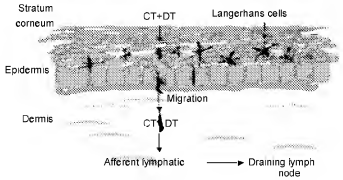
The skin is equipped with a powerful immune system located in the epidermis, which is bypassed by common immunisation techniques that use injection. Thus, when needles are used for an im. injection, they pass through the network of professional antigen presenting cells in the epidermis and target tissues where immune cells are relatively sparse. The superficial nature of the major component of the 'skin immune system', epidermal Langerhans cells (**Figure 1**), and their sheer number (up to 1000 cells/mm^2) [13], suggests that the protective outer layer of dead skin is an imperfect barrier, commonly traversed by organisms. The fragile skin represents the body's greatest exposure to the hostile microbial world and thus needs a protective layer of immune cells. Although the immune mechanisms engaged in response to immunologic challenges at the skin surface are highly complex, we hypothesised that this natural provision for biological defence could be manipulated for the purposes of vaccination using simple penetration enhancement techniques.

We have recently shown that topical administration of an adjuvant and antigen to hydrated, but otherwise intact skin can induce potent systemic immune responses [1,2]. TCI induces priming and secondary humoral antibody responses [2], confers protection against toxin challenge [2,6] and can be induced without signs of local or systemic toxicity. The immune response induced by TCI is highly dependent on the presence of an adjuvant, such as cholera toxin (CT) or heat labile enterotoxin from *Escherichia coli* (LT) [14]. CT and LT are members of a widely used class of mucosal adjuvants, the bacterial ADP-ribosylating exotoxins (bAREs), known to be among the most potent stimulators of humoral and T-cell responses [14,15]. Although the adjuvant mechanisms by which the bAREs act as adjuvants are unclear [16,17], the simple admixture of CT with a poorly immunogenic co-administered antigen results in high levels of antibodies and CD4+ [16,18] or CD8+ [19] T-cell responses to the co-administered antigen.

Hydration of the skin is known to disrupt the barrier to adsorption presented by the stratum corneum [20,21]. However, it has been held that penetration of the skin by any entity is largely restricted to molecules of relatively small size, up to 500 Da [22,23]. Conversely, CT is a relatively large molecule (86,000 Da) that would not be expected to penetrate the skin. However, we have found that a variety of vaccine antigens and adjuvants are able to induce potent immune responses following hydration and topical vaccine application, and thus hypothesised that hydration is sufficient to allow large molecules access to the superficial layer of the skin, where they may encounter Langerhans cells.

The term 'skin immune system' encompasses both cellular and humoral constituents found in the skin, which comprise a highly active immunologic surveillance and effector system [24]. The immune surveillance is conducted by antigen-presenting cells that migrate into the skin from the bone marrow, phagocytose antigen and migrate into the draining lymph node [25]. The predominant antigen-presenting cell, the epidermal Langerhans cell, has been shown to possess a constant level of transit from the skin to the draining lymph node [26] which is greatly amplified by appropriate 'danger' signals, such as contact sensitisers [27], LPS [28] or cytokines [29]. Two locally produced cytokines, TNF-α and IL-β appear to play dominant roles in activation and subsequent migration of LCs out of the skin [30,31]. The presence of an adjuvant, such as CT, in the

Figure 2: Illustration of the operating hypothesis for transcutaneous immunisation. Antigen and adjuvant placed on the skin penetrate into the superficial layers of the epidermis through simple hydration. Langerhans cells phagocytose antigen (DT) and are activated by CT, possibly by the induction of TNF-α and IL-1β. Activated Langerhans cells migrate out of the skin and into the draining lymph node, where antigen presentation of DT to T-cells occurs, resulting in a subsequent systemic immune response to DT.



epidermal milieu may engage these mechanisms; such considerations are topics of active research.

Although the ability of CT to induce an immune response when applied to the skin was unexpected, other delivery techniques that appear to utilise the immunologic components of the epidermis for the purposes of vaccination have been well described. Intradermal delivery of vaccine antigen has long been held as a superior anatomical target that results in potent immune responses without signs of local inflammation [32,33]. However, intradermal immunisation is technically difficult to perform, is painful and relies on needle-based delivery. Bolistic delivery (gene gun) of DNA encoding for vaccine antigens into the skin clearly recruits the skin immune system and induces similar potent systemic immune responses [4,34]. Immune responses to topically applied contact sensitises are also well described, and the subsequent induction of specific T-cells result in immune-mediated damage to the skin [35]. Thus, TCI appears to utilise an immunologic milieu that has been demonstrated to result in powerful immune manifestations in response to stimulation by other means.

While the mechanisms engaged in TCI are yet to be fully characterised, it has been helpful to apply a working hypothesis to guide experimentation. We have hypothesised that hydration of the skin allows superficial penetration of the stratum corneum by adjuvants such as CT. The *LCs* then phagocytose the antigen, and the adjuvants activate LCs, which then migrate to the draining lymph node where the antigen is presented to T-cells for the induction of an immune response (**Figure 2**). Thus, the theoretical basis of TCI has its foundations in well-established scientific paradigms relating to skin penetration, Langerhans cell biology and the role of adjuvants in immune response augmentation. While transdermal drug delivery also utilises hydration in conjunction with patch delivery of drugs, the goal generally is to access the deeper layers of the skin, especially the highly vascular dermis, in order to achieve systemic levels of the drug. We have coined the term TCI to distinguish our target for vaccine antigen and adjuvant delivery, the superficial layer of the skin, and thereby evoke the implications of utilising the elements of the Skin Immune System.

4. Immunisation procedure

TCI is a simple technique that, in its final clinical manifestation, will involve application of a patch to a vaccinee on a convenient skin location. The use of TCI in mice is more laborious, as they are hairy and do not tolerate patches or remain stationary for simple topical application. However, we have successfully immunised BALB/c, C57BL6, C3H, A/J, and outbred (Swiss Webster) mice, as well as guinea-pigs, NZ White Rabbits, cats and dogs. In general, mice are carefully shaved to avoid nicking on the dorsum with a No. 40 clipper and rested for 48 h. The mice are anaesthetised with ketamine:xylazine for 0.5 - 2 h during the immunisation procedure to prevent grooming. The skin is wetted with water for several minutes and then 25 - 100 µl of immunising solution is placed on the shaved skin over a 1 - 2 cm^2 area. The mice are then extensively washed with lukewarm tap

Table 1: Antibody responses to CT, CT-subunits and other bAREs.

Immunising antigen	Antibody specificity	Serum IgG (ELSA Units)	SEM
CT	Anti-CT	39,828	(17,826 - 44,838)
CTB	Anti-CTB	7480	(3756 - 14,896)
rCTB	Anti-CTB	9324	(5271 - 13,372)
CTA	Anti-CTA	0	0
LT	Anti-LT	22,461	(20,262 - 27,167)
ETA	Anti-ETA	3758	(1951 - 77,240)
BSA	Anti-BSA	0	0

Balb/C Mice (n = 5) were immunised with 100 μg of antigen at 0, 3 weeks. Antibodies were measured by ELISA at 6 weeks. The results are reported as the geometric mean ± SEM of individually assayed sera in ELISA. Reprinted with permission of the American Society of Microbiologists.

water, patted dry and washed again. The washing is performed to avoid immunisation by grooming. Numerous control experiments have ruled out a possible role for ingestion of the adjuvant/antigen mixture in the immunisation process. No adverse effects from the shaving, anaesthesia, immunisation, or washing procedures are generally observed.

5. TCI induces potent immune responses to bAREs

The earliest observations of systemic antibody responses to topically applied immunising solutions were seen with the use of bAREs. As shown in **Table 1**, CT administered alone by the transcutaneous route induces high levels of anti-CT antibodies. A protein, such as bovine serum albumin (BSA), is not immunogenic when given under the same conditions. CT is a member of the bAREs, which are characterised by an A:B subunit structure [15]. In the case of CT, the A-subunit contains the ribosyltransferase activity thought to account in large part for its adjuvant activity [16,36] and the B-subunit which binds to surface gangliosides. The CT B-subunit has also been shown to have mucosal adjuvant activity in some settings [37,38]. Similar to CT, the purified B-subunit of CT (CTB) induces a strong antibody response when given by TCI, but is generally of a lower magnitude. Although purified preparations of CTB subunit may contain small amounts (< 0.01%) of the enzymatic cholera toxin A subunit (CTA), rCTB, which lacks CTA entirely, was equally immunogenic on the skin when compared to purified CTB. In contrast, purified CTA induces little or no anti-CTA IgG titres, suggesting that B-subunit activity is important for TCI. Topical application of LT, which is very similar to CT in size, amino acid sequence and receptor binding activities [15], induces a similarly high anti-LT antibody response in the sera. Pseudomonas exotoxin A (ETA), which contains both the A and B domains that characterise the bAREs on the same peptide, and neither bears structural homology to LT or CT, nor shares the surface binding affinities [15], also induces a transcutaneous anti-ETA antibody response. Thus, as a group, the bARE family of toxins appears active when applied as antigens by the transcutaneous route.

6. TCI induces classic immune responses to vaccination

The role of an adjuvant is essential to the induction of an immune response utilising TCI [1]. CT and the closely related heat labile enterotoxin from *E coli* (LT), have been extensively studied as mucosal adjuvants, providing an enormous fund of knowledge for the development of TCI [16,17,36]. CT or LT, simply mixed with a vaccine antigen and administered into the nasal mucosa, induces an immune response to the vaccine antigen, that otherwise would be absent or weak. The strength of the immune enhancement contributed by the use of CT or LT has established these adjuvants as the gold standard for mucosal adjuvants [17]. However, the perceived mucosal toxicity of CT and LT has limited widespread use of these proteins as human vaccine components and adjuvants and has led to mucosal strategies using genetically altered 'non-toxic' mutant toxins [39,40] and purified B-subunits [37]. However, the finding that

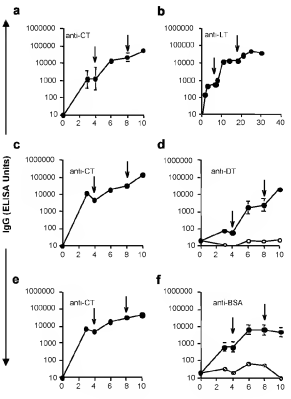


Figure 3: Kinetics of the IgG (H+L) antibody response to CT (a, c, e), LT (b), DT (d) or BSA (f) in animals immunised and boosted (arrows) by the transcutaneous route. BALB/c mice (n = 5) were immunised with (a), CT alone (100 μg), (b) LT alone (100 μg), (c, d) CT + DT (100 μg CT, 100 μg DT), (d), DT alone (100 μg DT), (e, f) CT + BSA (100 μg CT, 200 μg BSA) or (f) BSA alone (200 μg BSA). Grey circles in d, f indicate antibody levels to DT or BSA respectively in mice vaccinated without using CT as adjuvant. Antibodies were measured by ELISA at multiple time points. The results are reported in ELISA units, the inverse dilution at which the OD = 1 and shown as the mean ± SEM. Similar results were obtained in two independent experiments. Reprinted with permission by the American Society of Microbiologists.

application of CT to the skin induces robust immune responses, without the local or systemic toxicity that accompanies its use by the oral, nasal or parenteral routes, suggests that TCI may facilitate the safe use of native CT with its unmatched potency as an adjuvant [2,7].

The use of CT as an adjuvant on the skin results in classic priming and secondary antibody responses to co-administered antigens. In **Figure 3** (**a**,**b**), mice immunised with CT or LT alone, and subsequently boosted, demonstrate a typical secondary antibody response to the toxins. When CT is co-administered with the vaccine antigen diphtheria toxoid or the protein BSA, minimal priming antibody responses are seen to the co-administered antigens (**Figure 3d, 3f**). However, after boosting immunisations, classic secondary antibody responses are seen. In parallel, anti-CT priming and secondary responses occur (**Figure 3c, 3e**). Importantly, the humoral response to the adjuvant has been shown not to interfere with the response to co-administered antigen, and multiple immunisations with the same adjuvant may be conducted [2]. The serum anti-CT antibodies may be used to confirm the proper delivery of the vaccine solution on the skin when CT is used as the adjuvant, as successful immunisation will always result in serum anti-CT antibodies [2]. Thus, high levels of antibodies to vaccine antigens can be induced, similar to those induced by priming and boosting using im. immunisation with alum or intranasal immunisation with LT or CT as adjuvants. Most vaccines acquire their protective levels of antibodies through boosting regimens, and it appears that TCI similarly induces memory responses that can be readily boosted.

Antigen-specific T-cells would be expected to underlie the secondary antibody responses seen in response to TCI. Mice immunised on the skin with DT using CT as adjuvant have been found to have proliferative responses in the spleen and draining lymph (**Table 2**). Consistent with T-cell help, these proliferative responses to DT appear to be due to $CD4^+$ cells. Thus, it appears that TCI induces both secondary antibody responses and T-cell help that underlie the ability to achieve high levels of antibodies.

7. Optimisation of the immune response to TCI

The earliest experiments conducted using TCI were performed using empiric antigen doses, without reference to optimised immunisation by other routes. The working hypothesis for TCI suggests that potent immune response, comparable or superior to immune response induced by standard routes should be feasible, as this technique uses gold standard adjuvants to target antigen presenting cells that have been termed 'nature's adjuvants' (Iangerhans cells) [13]. Optimisation experiments have suggested that TCI can elicit responses comparable in magnitude to

Table 2: Antigen-specific T-cell proliferation induced by topical application of CT and DT to the skin.

Immunisation	Source of cells	*In vitro* stimuli		Fold rise
		Media (cpm)	DT (cpm)	
CT	Inguinal LN	3700	4407	1.2
CT and DT	Inguinal LN	6061	55056	9.1
CT and DT	CD4+ T-cells Spl	236	9612	40.7

DT and/or CT was applied to the skin of C3H/HeJ mice (n = 5) at 0, 4, and 8 weeks. Spleen and draining lymph nodes (LN) were removed 11 weeks after the third immunisation, cells from individual mice pooled within each group, and single cell suspensions prepared. Cells from the site draining the site of immunisation (inguinal node) and CD4+ T-cells that were column purified from a whole spleen cell population were stimulated *in vitro* with media or antigen (DT; 10 µg/ml) for 96 h. 3-H thymidine incorporation was measured during the last 12 h of culture. Cultures were assayed in triplicate and the average cpm is reported. The fold rise in proliferation was calculated as the ratio of cpm in cultures stimulated with DT *versus* in media alone.

those seen in response to established routes of immunisation, enhance responses by simple, practical skin manipulation, achieve similar results with less adjuvant and antigen and immunise in a short period of time.

Previous experiments have shown that simple alcohol swabbing of the skin, prior to application of the immunising solution, can enhance the antibody response to CT and provide a more uniform immune response [7]. In another experiment, the skin was alcohol swabbed and hydrated prior to immunisation with CT and DT (**Table 3**). The anti-DT antibody responses induced by TCI were compared to anti-DT responses elicited by im. injection with alum and intranasal (in.) immunisation using LT as adjuvant (**Table 3**). The response elicited by TCI was equivalent or slightly superior to the responses induced by the im. or in. immunisations, although the doses used for TCI were higher.

In a subsequent experiment, a variety of combinations of adjuvant and antigen were used in mice to determine whether the adjuvant or antigen doses could be lowered. Using 100 µg of antigen (diphtheria toxoid) and 100 µg of antigen (CT) as the standard dose, mice were dosed with various combinations of 10 µg of antigen and/or adjuvant, and the anti-DT responses were compared to the standard dose (**Figure 4**). Not surprisingly, the mice that received 10 µg of adjuvant and 100 µg of antigen exhibited anti-DT antibody responses which were equivalent to the mice receiving 100 µg of antigen and adjuvant. This study suggests that far lower doses of adjuvant may be used in the induction of immune responses using TCI, and that in combination with simple skin manipulation, doses used for im. or in. routes may be sufficient for immune responses induced by TCI.

The duration of time required for induction of an immune response *via* TCI is not clear, but if hydration of the skin and passive diffusion of the antigen into the epidermis are the underlying physical phenomena required for immunisation, then a short period of immunisation may be feasible. To determine the minimum time required for TCI, the immunising solution was applied for 15, 30, 60 or 120 min and the animals were extensively washed as described. Clearly, after 15 min of application the animals were immunised and by 30 - 60 min, maximal immune responses were obtained (**Figure 5**). This study suggests that clinical application strategies could anticipate short immunisation periods that may fit into a clinic visit for routine healthcare.

8. Expert Opinion

TCI is a new method for inducing immune responses. Our initial observations, that an adjuvant and antigen topically applied to the skin using simple penetration techniques such as skin hydration, will almost certainly stimulate further investigation. The observation that TCI is feasible has arrived at a time when vaccine policy bodies concerned with widespread implementation of vaccines, such as the WHO and the CDC, have determined needle-free delivery of vaccines to be a high priority. TCI has become a new candidate strategy that may address WHO/CDC concerns. The simplicity of TCI suggests that it can be implemented relatively quickly on the time scale of vaccine development. In particular, the use of current formulations seems readily applicable to a patch

Table 3: Antibody responses to DT administered by different routes.

Immunising antigen/adjuvant	Route of immunisation	Anti-DT IgG (ELISA units)	Range
DT+CT	Transcutaneous	135,792	(86,552 - 212,575)
DT+Alum	Intramuscular	95,936	(24,675 - 238,904)
DT+CT	Intranasal	85,492	(65,944 - 109,772)

Comparative immune responses to diphtheria toxoid administered by different routes. BALB/c mice (n = 5) were immunised with 50 μg of diphtheria toxin in commercially available DTaP using either 100 μg of CT as adjuvant on the skin, 1 μg of CT as adjuvant in. or 3 μg of alum as adjuvant im.. Mice were immunised three times. Anti-DT IgG titres were assessed by ELISA on serum collected 3 weeks after the last immunisation. Titres in prebleed serum from the same batch of animals were 20 EU. Results are reported in ELISA Units, which are defined as the inverse dilution of the sera that yields an OD of 1.0 at 405 nm. The geometric mean and range of 5 animals is shown.

Figure 4: Reduction of adjuvant dose to 10 μg does not affect induction of anti-DT titres following immnisation on the skin. C57BL/6 mice (n = 3 - 9) were immunised with CT (10 or 100 μg) and DT (10 or 100 μg) on the skin at 0, 4, and 8 weeks. Anti-DT IgG titres were assessed by ELISA on serum collected 12 weeks after the primary immunisation. Titres in pre-bleed serum from the same batch of animals were = 20 EU. Results are reported in ELISA Units, which are defined as the inverse dilution of the sera that yields an OD of 1.0 at 405 nm.

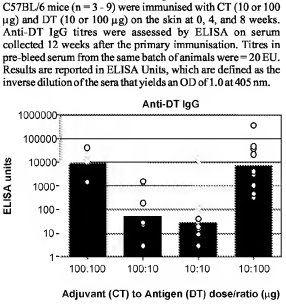
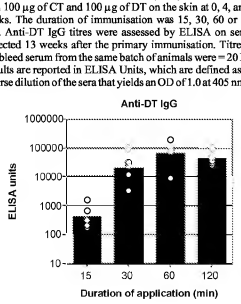


Figure 5: Achievement of maximal anti-DT titres in animals immunised for 30 min. C57BL/6 mice (n = 5) were immunised with 100 μg of CT and 100 μg of DT on the skin at 0, 4, and 8 weeks. The duration of immunisation was 15, 30, 60 or 120 min. Anti-DT IgG titres were assessed by ELISA on serum collected 13 weeks after the primary immunisation. Titres in pre-bleed serum from the same batch of animals were = 20 EU. Results are reported in ELISA Units, which are defined as the inverse dilution of the sera that yields an OD of 1.0 at 405 nm.



vaccination and, along with the possibility of boosting individuals primed by other routes, suggest that TCI booster trials will be the first development in clinical trials. Other problems that may be addressed through a simple patch application of vaccines, include vaccines that require multiple or annual boosting, multivalent vaccines and new vaccines to be added to current immunisation schedules. In summary, TCI is an eminently practical procedure that appears to provide unprecedented access to the innate immune system. Thus, manipulation of the immune response *via* the TCI is a highly promising concept and should be a fruitful avenue of future research.

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United States Patent [19]

Gross et al.

[11] Patent Number: 5,848,991

[45] Date of Patent: Dec. 15, 1998

[54] **INTRADERMAL DRUG DELIVERY DEVICE AND METHOD FOR INTRADERMAL DELIVERY OF DRUGS**

[75] Inventors: **Joseph Gross**; **John Gerard Kelly**, both of Dublin, Ireland

[73] Assignee: **Elan Medical Technologies Limited Athlone, Co.**, Westmeath, Ireland

[21] Appl. No.: **843,146**

[22] Filed: **Apr. 25, 1997**

Related U.S. Application Data

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[52] **U.S. Cl.** **604/140**; 604/890.1

[58] **Field of Search** 604/140–143, 604/145, 147, 890.1, 892.1, 66, 131–134

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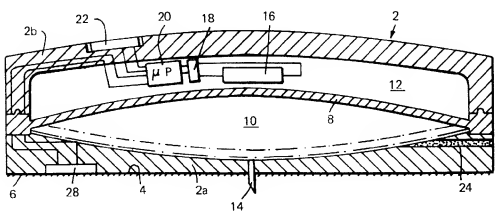
Primary Examiner—Jeffrey R. Jastrzab
Attorney, Agent, or Firm—Kathleen L. Maher

[57] **ABSTRACT**

An intradermal drug delivery device for delivering a liquid drug to a subject via the subject's skin includes a housing having a lower surface provided with an adhesive coating for adhering the housing to the subject's skin. An expansible-contractible chamber within the housing defines a reservoir which is expanded upon being filled with the drug and contracted to dispense the drug. A hollow needle extends through the lower surface of the housing and has an inner end which communicates with the reservoir and an outer end which projects outwardly of the housing a short distance to penetrate through the epidermis and into the dermis of the subject's skin when the housing is adhered thereto. The device permits delivery of drugs of relatively large molecular size and at slow rates which can be precisely controlled. A method of delivering a liquid drug intradermally includes adhering the intradermal delivery device to the skin of the subject and activating the means for actively discharging the at least one drug from the reservoir to the subject's skin vi the needle.

32 Claims, 7 Drawing Sheets



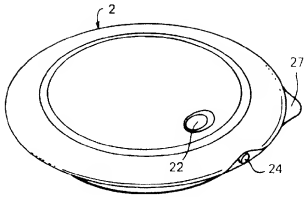


FIG. 1

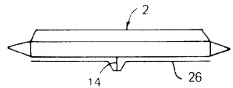


FIG. 2

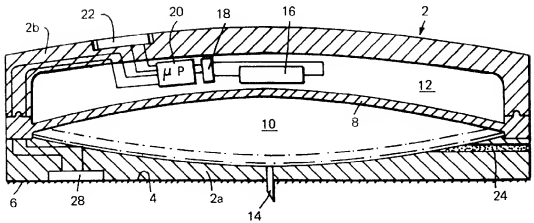


FIG. 3

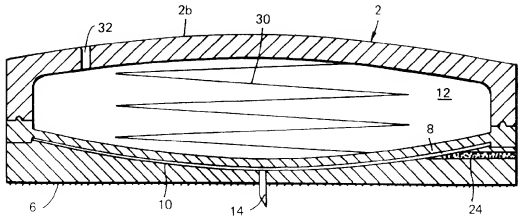


FIG. 4

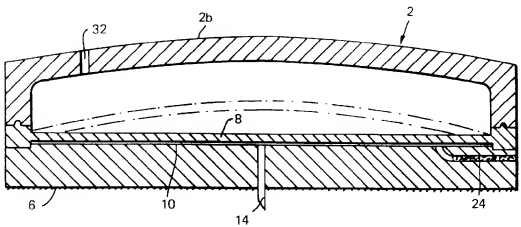


FIG. 5

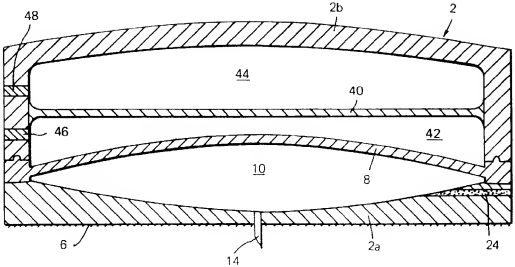

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FIG. 6

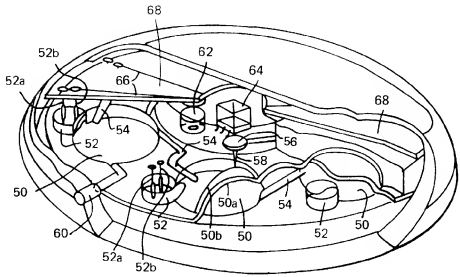
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FIG. 7

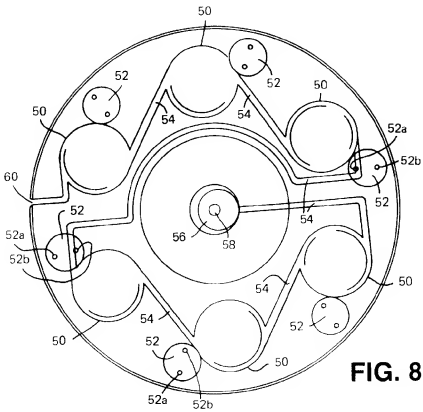
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FIG. 8

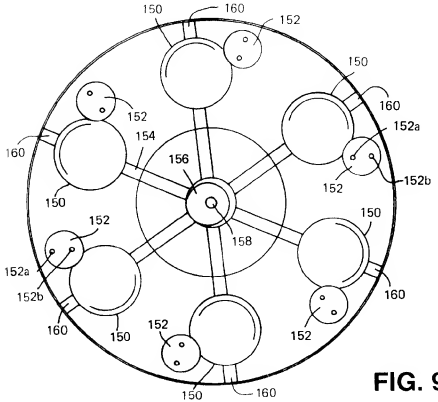
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FIG. 9

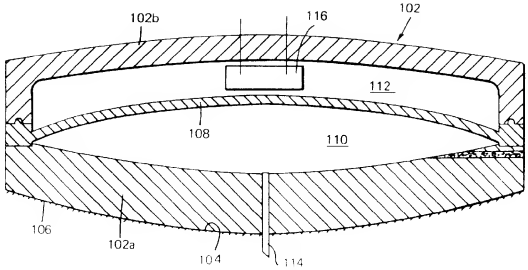

FIG. 10

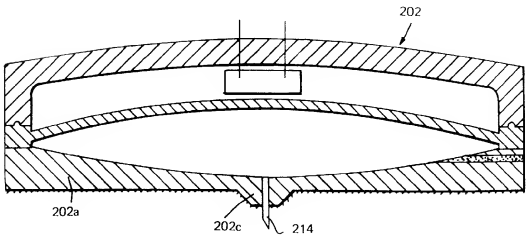

FIG. 11

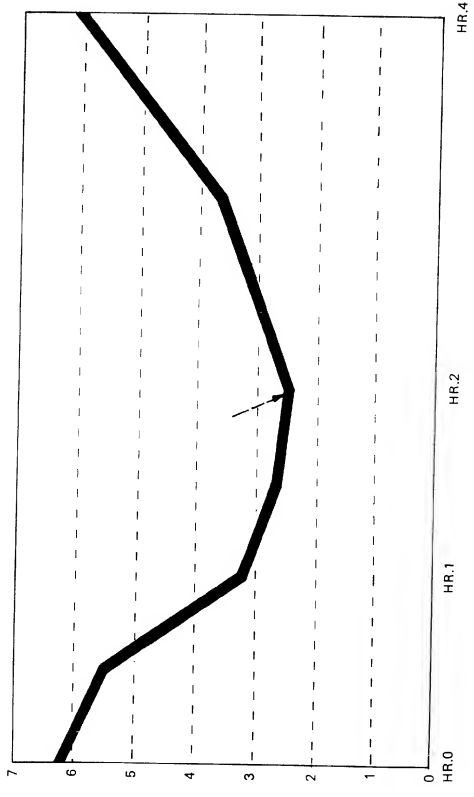

FIG. 12

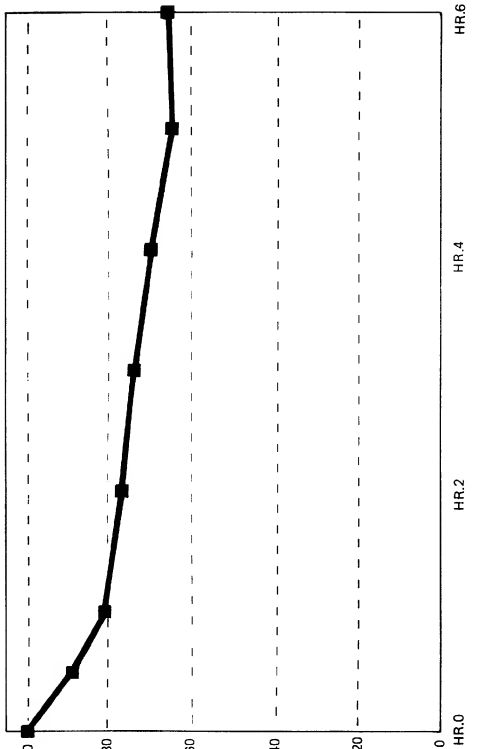

FIG. 13

INTRADERMAL DRUG DELIVERY DEVICE AND METHOD FOR INTRADERMAL DELIVERY OF DRUGS

RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08/601,450, filed Feb. 14,1996, which is division of patent application Ser. No. 08/183,482 filed Jan. 18, 1994 now U.S. Pat. No. 5,527,288, which is a continuation-in-part of our patent application Ser. No. 07/981,652 filed Nov. 25, 1992, now U.S. Pat. No. 5,279,544, which in turn is a continuation-in-part of our patent application Ser. No. 07/850,595 filed Mar. 13. 1992 now abandoned, which in turn is a continuation-in-part of our patent application Ser. No. 07/627,104, filed Dec. 13, 1990, now U.S. Pat. No. 5,156,591.

BACKGROUND OF THE INVENTION

The present invention relates to drug delivery devices, and in particular to an intradermal drug delivery device for delivering a liquid drug to a subject via the subject's skin.

One type of transdermal drug delivery device is in the form of a patch applied to the subject's skin and containing a drug penetrating the skin by osmosis and/or by a controlled mass transport phenomenon such as iontophoresis. Simple patches, however, provide no control, or limited control, of the rate of drug delivery, which depends on skin conditions, the nature (particularly molecular size) of the drug to be delivered, and the like. Iontophoresis devices are also not entirely satisfactory in their ability to deliver large molecules and to control the rate of delivery thereof. All such devices are limited by the barrier function of the skin.

Another transdermal drug delivery device is described in International Patent Publication WO93/17754. In one embodiment this device comprises a housing containing a liquid reservoir and a drug delivery body carried by the housing and engageable with the subject's skin. The drug delivery body carries a plurality of hollow needles (of which there are preferably at least fifty) having an outer diameter of the order of 1 mm, which needles are designed to pierce the outer layer of dead cells (the stratum corneum) of the skin, thereby enhancing the penetration of the drug through the skin.

However, certain disadvantages are associated with this method of drug delivery. Firstly, there is a risk of considerable pain and traumatization of the skin associated with the application of the particular array of needles. Secondly, the drug may leak out around the entry point of each needle as a result of the pressure being applied to assist the delivery of the drug. A film of liquid drug covering the area of application may cause irritation for subjects with sensitive skin; certain drugs may aggravate this irritation. The leakage also results in a lower efficiency of drug delivery. Thirdly, it can be difficult to ensure that the device is correctly applied with the tips of the needles penetrating the stratum corneum. The skin has a natural resilience and elasticity. The device is pressed onto the skin such that the entire area of the needle arrangement depresses the surface of the skin, even when considerable pressure is applied. For this reason, an extra degree of pain is associated with the correct application of the device, due to the amount of force needed to properly pierce the stratum corneum with all of the needles.

BRIEF SUMMARY OF THE INVENTION

According to the present invention, there is provided an intradermal drug delivery device for delivering a liquid drug to a subject via the subject's skin, comprising: a housing having a lower surface for application to the skin of the subject; means for affixing the housing in position with the lower surface in contact with the subject's skin; a drug reservoir within the housing; a single hollow needle associated with the drug reservoir extending through the lower surface, having an inner end communicating with the drug reservoir and an outer end projecting outwards a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is pressed against the skin; and means for actively discharging the drug from the reservoir to the subject's skin via the needle; the lower surface being shaped such that when pressed against the skin, a substantial proportion of the pressure applied to the skin is directed through the needle tip; and the needle having an outer diameter 0.2 mm or less.

According to the preferred embodiments described below, the needle projects outwardly of the housing approximately 0.3–3.0 mm, most preferably 0.3–1.0 mm, and has an outer diameter of 0.1–0.2 mm and an inner diameter of 0.05–0.075 mm.

As will be described more particularly below, such an intradermal drug delivery device permits the delivery of a variety of drugs including drugs of relatively large molecular size, and at slow rates which can be precisely controlled.

According to further features of the invention described below, the drug reservoir may be an expansible-contractible chamber which is expanded upon being filled with the drug and is contracted to dispense the drug therefrom at controlled rates by the means for actively discharging the drug. These means can include an electrically-controlled gas generator, such as an electrolytic cell, a prestressed spring or membrane, or osmotic means to provide for osmosis between a pure water compartment and a saline compartment included within the housing.

According to another aspect of the invention, there is provided a drug delivery device having a plurality of drug reservoirs within the housing, all drug reservoirs communicating with an outlet cavity with which the single hollow needle also communicates, and means such as electrical means for individually controlling the feeding, of drug from the plurality of reservoirs to the outlet cavity.

Further features and advantages of the invention will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. **1** illustrates one form of an intradermal drug delivery device constructed in accordance with the present invention;

FIG. **2** is a side elevational view of the device of FIG. 1;

FIG. **3** is an enlarged longitudinal sectional view of the device of FIG. 1;

FIGS. **4**, **5** and **6** are longitudinal sectional views illustrating other intradermal drug delivery devices constructed in accordance with the invention;

FIG. **7** is a diagrammatic view illustrating a multi-reservoir intradermal drug delivery device in accordance with the invention;

FIG. **8** is a top plan view more particularly illustrating the internal structure of the device of FIG. **7**;

FIG. **9** is a view corresponding to that of FIG. **8**, but showing a modification wherein the drug reservoirs are connected in parallel with the outlet cavity rather than in series as in FIG. **8**;

FIGS. **10** and **11** illustrate two further variations in the construction of the device; and

FIGS. **12** and **13** show delivery characteristics of insulin and salmon calcitonin, respectively, from a device constructed in accordance with the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The device according to the invention overcomes the disadvantages indicated above for the following reasons: Firstly, since only a single needle is generally used, only a single point of entry is associated with the application of the device, eliminating most of the pain and trauma resulting form the application of the device. In addition, the extremely narrow diameter of the single needle allows the application to be virtually painless and minimally invasive.

Secondly, the amount of leakage is diminished to a very large extent, if not totally. The delivery is far more controlled as a result. The leakage is reduced for two reasons: (i) the drug is delivered below the epidermis (and not just to below the stratum corneum); and (ii) only a single point exists at which leakage might occur.

Thirdly, the shape of the lower surface results in a substantial proportion of the pressure being directed through the needle tip. If the device is not correctly shaped, too much pressure may be directed through the lower surface so that the skin is stretched by the surface of the device and not the needle. According to the invention, the needle must provide sufficient pressure to stretch and pierce the epidermis, i.e., the elasticity of the skin must be directed against the needle. It should be noted that the effective pressure (force applied to the housing per unit area of skin contact) is, for a given force, far higher for the device according to the invention, since the effective area of application is diminished approximately fifty-fold when only one needle is applied as opposed to 50 needles, and is further reduced as a result of the narrow diameter needle used.

Since the intradermal device of this invention delivers the drug below the epidermis, i.e., to the interface between the epidermis and the dermis or to the interior of the dermis or subcutaneously, many of the problems of transdermal application are non-existent; the drug is delivered directly to a capillary-containing tissue and has no barriers to pass through before entering the vascular system.

Preferably, the means for holding the housing in position comprises a pressure-adhesive coating, such as an acrylate adhesive, on the lower surface thereof. When the device is pressed against the skin, the needle penetrates the epidermis and the pressure-adhesive coating affixes the lower surface to the skin. A single-step, painless and trauma-free application is thus provided by the invention. Additionally or alternatively, the device may be held in position by a strap or bracelet.

According to one embodiment of the invention, the lower surface of the housing has a convex shape and the hollow needle extends from the center of the convexity. Alternatively, the lower surface of the housing is provided with a protuberance from which the needle projects. In a further alternative, the lower surface of the housing is of a conical shape and the hollow needle extends from the apex of the cone. In a further embodiment, the lower surface of the housing can have a convex shape and also be provided with a protuberance from which the needle projects.

In each case, the needle is positioned to engage the skin directly so that it pierces the skin before a large part of the surface has made contact. In effect, parts of the surface distal from the needle are held back from the skin as a consequence of the shape of the lower surface. For this reason, much of the pressure which might have been applied by the surface of a flat device is instead directed through the needle tip.

The device may however have a flat surface provided that the size of the device or the shape and elasticity of the skin to which the device is to be applied enables a substantial portion of the pressure to be directed through the needle tip.

Preferably, the needle projects outwards of the housing by approximately 0.3–3.0 mm, most preferably 0.3–1.0 mm, and has an outer diameter of 0.1–0.2 mm and an inner diameter of 0.05–0.075 mm. Such a needle is relatively painless to apply, causes little or no trauma to the skin and yet allows precisely controllable delivery of a liquid drug, including drugs of relatively large molecular size.

Preferably, the reservoir is in the form of an expansible-contractible chamber which is expanded when filled with the drug and which can be contracted to dispense the drug therefrom.

Further, preferably, the drug reservoir, when filled, has a volume of 0.2–10.0 ml or larger, more preferably 0.3–6.0 ml, most preferably 0.5 to 3.0 ml.

Further, preferably, the means for actively discharging the drug comprises an electrically controlled gas generator within the housing for generating a gas to contract the drug reservoir in order to discharge the drug therefrom.

Such an intradermal delivery device provides precise control over the rate of delivery of the drug; in particular, it allows the drug to be delivered at precisely controllable slow rates. The use of a narrow needle is also advantageous for achieving slow rates of delivery, while still allowing the delivery of a variety of drugs, including those of relatively large molecular size.

Suitably, the gas generator is an electrolytic cell. In a preferred embodiment of the invention, the device further comprises a start button which is depressible in order to activate the means for actively discharging the drug from the drug reservoir, such as a start button which energizes a gas generator. Thus, the device may be supplied and stored for an indefinite period of time and yet be immediately activated when required.

Suitably, the device comprises an electronic circuit for controlling the time and rate of gas generation, thereby controlling the discharge of the drug from the drug reservoir. Preferably, the electronic circuit comprises a microprocessor which is programmable with respect to the time and rate of gas generation. For instance, the microprocessor can be programmed to deliver the liquid drug in a continuous infusion, in a pulsatile manner or in intermittent doses as well as in response to input from the subject, such as patient controlled analgesia.

It is thus possible to choose or devise a dosage regime which will suit the requirements both of the individual patient and of the drug to be delivered. For example, the device may comprise a microprocessor which controls the delivery such that the rate of delivery is varied during a 24 hour cycle as is necessary due to the differing requirements of drug dosage during periods of activity, inactivity and sleep, and taking account of the subject's requirements in relation to food intake.

Alternatively, the subject might be provided with separate daytime and nighttime devices, each having a different electronic circuit for controlling the time and rate of drug delivery.

It may be desirable to automatically deliver certain drugs only when required by the subject, either by patient activation or passively, such as by a feedback mechanism. In such a case, there is provided a device wherein the housing further includes a sensor (feedback) for detecting a condition in the body of the subject and for controlling the delivery of the drug in response thereto. The sensor may be, for example, a temperature sensor, a pulse rate sensor, a blood glucose sensor, a blood pressure sensor or a pH sensor.

Thus, where a device is intended to deliver a fever-reducing drug, for example, it might be provided with a temperature sensor such that a detected increase in body temperature above a certain value would activate the drug delivery or increase the rate of drug delivery.

The sensor may rest against the skin, may be inserted through the skin, or may be within the device and separate from the skin.

According to one embodiment of the invention, the housing includes a plurality of drug reservoirs, each reservoir being contractible by a separate gas generator and communicating with an outlet cavity with which the single hollow needle also communicates. In one such embodiment, all of the drug reservoirs communicate in series with the outlet cavity. In an alternative embodiment, all of the drug reservoirs communicate in parallel with the outlet cavity.

Including a plurality of drug reservoirs provides for considerable variations in the amounts of drug which can be delivered, in the rates at which drug can be delivered and in the number of drugs which can be delivered by the same device. The provision of a plurality of reservoirs allows the device to be used in a range of situations for which a single reservoir device would be unsuitable.

A preferred embodiment of a device which is to deliver more than one drug has a housing which includes a plurality of drug reservoirs, each having a single hollow needle associated therewith. Such a device is especially suitable when the drugs are not suitable to mix with one another or when they are to be delivered separately or sequentially.

In an alternative embodiment of a device according to the invention wherein the reservoir is in the form of an expansible-contractible chamber, the means for actively discharging the drug comprises a spring which is stressed by the expansion of the drug reservoir upon filling it with a drug, and which tends to return to its unstressed condition to contract the reservoir and thereby to discharge the drug via the hollow needle.

In another alternative embodiment wherein the reservoir is in the form of an expansible-contractible chamber, the means for actively discharging the drug comprises a membrane which is stressed by the expansion of the drug reservoir upon filling it with a drug, and which tends to return to its unstressed condition to contract the reservoir and thereby to discharge the drug via the hollow needle.

Either of the last mentioned alternative embodiments provide for devices which can be reusable when provided with means for refilling the drug reservoir. This refilling may take place either upon removal of the device or in situ.

In another alternative embodiment of the device according to the invention, the means for actively discharging the drug comprises a deformable liquid-impermeable membrane and a rigid liquid-permeable membrane; one side of the deformable liquid-impermeable membrane defining one side of the drug reservoir; the opposite side of the deformable liquid-impermeable membrane and one side of the rigid liquid-permeable membrane defining a saline reservoir for receiving a saline solution: the opposite side of the rigid liquid-permeable membrane defining, with a rigid part of the housing, a pure water reservoir for receiving pure water to expand the saline reservoir by osmosis, thereby to contract the drug reservoir in order to dispense the drug therefrom via the hollow needle.

Such a device provides for a predictable and continuous delivery of the liquid drug, whose rate of delivery can be chosen according to the volume, concentration and nature of the saline solution used, since the expansion of the saline reservoir (and thus the contraction of the drug reservoir) depends on the osmotic pressure across the membrane separating the pure water reservoir from the saline reservoir.

Preferably, the device further comprises a membrane which is permeable to the liquid drug and impermeable to solid impurities, the membrane covering the inner end of the hollow needle. The advantage of the membrane covering the inner end of the hollow needle is to filter out solid particles to prevent clogging of the needle. Preferably, the pore size of this membrane may range from 0.2 μm to 1.0 μm.

The present invention also encompasses a method of delivering a biologically effective amount of a liquid drug intradermally to an animal subject, especially a human, comprising the steps of: (1) affixing an intradermal drug delivery device to the skin of the subject, the drug delivery device including a housing having a lower surface for application to the skin of the subject; means for affixing the housing in position with the lower surface in contact with the subject's skin; a drug reservoir within the housing and containing a biologically effective amount of at least one liquid drug; a single hollow needle associated with the drug reservoir having an outer diameter of 0.2 mm or less and extending through the lower surface and having an inner end communicating with the drug reservoir and an outer end projecting outwards a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is affixed to the skin; and means for actively discharging the at least one drug from the reservoir to the subject's skin via the needle; and (2) activating the means for actively discharging the at least one drug to deliver a biologically effective amount of the at least one drug to the subject.

As used herein, the term, "liquid drug," is meant to encompass any drug-containing fluid capable of being passed through the hollow needle in a controlled manner, such as a liquid, solution, gel or fine suspension. There is essentially no limitation on the type of liquid drug which can be used with the invention other than to exclude those liquid drugs which would be inappropriate to deliver to the subject intradermally or subcutaneously. Representative drugs include peptides or proteins, hormones, analgesics, anti-migraine agents, anti-coagulant agents, anti-emetic agents, cardiovascular agents, anti-hypertensive agents, narcotic antagonists, chelating agents, anti-anginal agents, chemotherapy agents, sedatives, anti-neoplastics, prostaglandins and antidiuretic agents.

Typical drugs include peptides, proteins or hormones such as insulin, calcitonin, calcitonin gene regulating protein, atrial natriuretic protein, colony stimulating factor, betaseron, erythropoietin (EPO), interferons such as α, β or γ interferon, somatropin, somatotropin, somatostatin, insulin-like growth factor (somatomedins), luteinizing hormone releasing hormone (LHRH), tissue plasminogen activator (TPA), growth hormone releasing hormone (GHRH), oxytocin, estradiol, growth hormones, leuprolide acetate, factor VIII, interleukins such as interleukin-2, and analogues thereof; analgesics such as fentanyl, sufentanil, butorphanol, buprenorphine, levorphanol, morphine, hydromorphone,

hydrocodone, oxymorphone, methadone, lidocaine, bupivacaine, diclofenac, naproxen, paverin, and analogues thereof; anti-migraine agents such as sumatriptan, ergot alkaloids, and analogues thereof; anti-coagulant agents such as heparin, hirudin, and analogues thereof; anti-emetic agents such as scopolamine, ondansetron, domperidone, metoclopramide, and analogues thereof; cardiovascular agents, anti-hypertensive agents and vasodilators such as diltiazem, clonidine, nifedipine, verapamil, isosorbide-5-mononitrate, organic nitrates, agents used in treatment of heart disorders, and analogues thereof; sedatives such as benzodiazepines, phenothiozines, and analogues thereof; narcotic antagonists such as naltrexone, naloxone, and analogues thereof; chelating agents such as deferoxamine, and analogues thereof; anti-diuretic agents such as desmopressin, vasopressin, and analogues thereof; anti-anginal agents such as nitroglycerine, and analogues thereof; anti-neoplastics such as 5-fluorouracil, bleomycin, and analogues thereof; prostaglandins and analogues thereof; and chemotherapy agents such as vincristine, and analogues thereof.

The Embodiment of FIGS. **1–3**

The intradermal drug delivery device illustrated in FIGS. **1–3** includes a housing **2** of disc or cylindrical configuration having a flat lower surface **4** coated with a pressure-sensitive adhesive **6** for adhering the housing to the skin of the subject to receive the drug. The interior of housing **2** includes a flexible liquid-impermeable membrane **8** defining an expansible/contractible chamber **10** between it and the lower section **2***a* of housing **2**, and a second expansible/contractible chamber **12** between it and the upper section **2***b* of the housing. Chamber **10** serves as a reservoir for receiving the drug to be delivered, whereas chamber **12** serves as a gas chamber for controlling the delivery of the drug from the reservoir **10**.

A hollow needle **14** extends through housing section **2***a*. The inner end of needle **14** communicates with the drug reservoir **10**, whereas the outer end of the needle projects outwardly of the flat surface **4** of the housing a short distance so as to penetrate the epidermis of the subject's skin when the housing is applied and adhered thereto. Preferably, hollow needle **14** projects outwardly of the flat surface **4** a distance of 0.3–1.0 mm, just sufficient to penetrate through the epidermis of the subject's skin. The outer diameter of the needle is preferably from 0.1–0.2 mm and its inner diameter is preferably from 0.05–0.075 mm. These dimensions permit a slow, precisely-controllable delivery of the drug from the drug reservoir **10**. The inner end of the hollow needle **14** may be covered by a filter membrane to prevent clogging particles from entering the needle.

The rate and time of delivery of the drug is controlled by a gas-generator **16** within the gas compartment **12**. Preferably, gas generator **16** is an electrolytic cell energized by a battery **18** and controlled by a microprocessor **20** when actuated by a START button **22** mounted on housing section **2***b*.

Housing section **2***a* further includes an injection plug **24** which may be pierced by a syringe needle, for example, in order to fill reservoir **10** with the drug to be dispensed. In addition, the adhesive coating **6** on the flat lower surface **4** of housing section **2***a* is normally covered by a protective strip **26** (FIG. **2**) which is peeled away when the device is to be used. Protective strip **26** preferably includes a tab extension **27** (FIG. **1**) to facilitate removing the strip.

Optionally, housing section **2***a* further includes a sensor **28** flush with surface **4** so as to be pressed against the skin of the subject when the device is applied to the subject and held by the adhesive coating **6**. For instance, sensor **28** may be a temperature sensor for sensing the temperature of the subject and for controlling microprocessor **20**, and thereby the dispensing of the drug, in response to the subject's temperature. Sensor **28** may be a pulse rate sensor for sensing the pulse rate of a subject and for controlling, via processor **20**, the dispensing of the drug in response thereto.

It will be seen that the device illustrated in FIG. **1–3** may be used in the following manner.

Drug compartment **10** is filled with the drug to be dispensed by injecting same via syringe needle through the injection plug **24**, thereby expanding the drug reservoir **10**, e.g., to the full-line position shown in FIG. **3**. Microprocessor **20** is preprogrammed according to the desired time and rate of delivery of the drug. Protective strip **26** is removed to expose the hollow needle **14**, and the device is then pressed against the subject's skin such that the needle **14** penetrates only through the epidermis. The adhesive coating **6** firmly adheres the device to the subject's skin.

When the delivery is to start, the START button **22** is depressed. This energizes the electrolytic cell **16** to generate a gas under the control of microprocessor **20**. This increases the pressure within gas chamber **12**, thereby deforming membrane **8** to contract the drug chamber **10**, to feed the drug from chamber **10** to the subject via the hollow needle **14** at a rate dependent on the rate of generation of the gas by the gas generator **16**. This rate is controlled by the microprocessor **20**.

The sensor **28** senses a predetermined condition of the subject and controls the delivery of the drug from reservoir **10** in accordance therewith. For example, sensor **28** may be a temperature sensor, for controlling the delivery of a fever-reducing drug; alternatively, it could be a pulse rate sensor or a blood pressure sensor for controlling the delivery of a drug appropriate to the sensed condition.

The Embodiment of FIG **4**

FIG. **4** illustrates a similar device as FIGS. **1–3**, and therefore corresponding parts have been identified by the same reference numbers. In the construction illustrated in FIG. **4**, however, the drug reservoir **10** is contracted to feed the drug via the hollow needle **14**, not by a gas generator as in FIGS. **1–3**, but rather by a spring **30** included in compartment **12** between the diaphragm **8** and the housing section **2***b*. The latter section is formed with an atmospheric vent **32**.

The device illustrated in FIG. **4** is used in the same manner as described above with respect to FIGS. **1–3**, except that, instead of effecting the delivery of the drug by means of a gas generator under the control of a microprocessor as in FIGS. **1–3**, the delivery of the drug is effected by spring **30** which is prestressed upon introducing the drug into reservoir **10** via the injection plug **24**.

The Embodiment of FIG. **5**

The device illustrated in FIG. **5** is similar to that of FIG. **4**, and therefore its corresponding parts are identified by the same reference numbers. In the device of FIG. **5**, however, instead of including a spring (**30**) which is stressed upon filling the chamber **10** with the drug, the diaphragm **8** is made of an elastic material which is prestressed when so filling the drug chamber, and thereby effects the delivery of the drug via the hollow needle **14**.

The Embodiment of FIG. **6**

FIG. **6** illustrates another device similar to those described earlier, and therefore the corresponding parts are also iden-

tified by the same reference numerals. In this case, however, the housing **2** includes not only the deformable liquid-impermeable membrane **8**, but also a rigid liquid-permeable membrane **40**. Thus, one side of the impermeable membrane **8** defines with housing section **2***a* the drug reservoir **10**, whereas the other side of membrane **8** defines, with one side of the rigid liquid permeable membrane **40**, a saline chamber **42**. The other side of the permeable membrane **40** defines with housing section **2***b* a pure water chamber **44**. Drug reservoir **10** may be filled as described above via the injection plug **24**. The saline chamber **42** may be filled via another injection plug **46**, and the pure water chamber **44** may be filled via another injection plug **48**.

It will be seen that when the three chambers **10**, **42** and **44** are filled as described above, water from chamber **44** will permeate by osmosis through membrane **40** into the saline chamber **42**, thereby expanding that chamber and contracting the drug reservoir **10**, forcing the drug out through the hollow needle **14**.

The Embodiment of FIGS. **7** and **8**

FIGS. **7** and **8** illustrate a device similar to that of FIGS. **1–3**, except that the device includes a plurality of separate drug reservoirs **50** (six being shown in FIG. **8** for example), each individually controlled by a gas generator **52**. All the drug reservoirs are connected in series via conduits **54** to a central outlet cavity **56** with which the hollow needle **58** communicates. An injection plug **60** may be used for filling all the reservoirs **50** in series.

Each of the gas generators **52** is a separate electrolytic cell including a pair of electrodes **52***a*, **52***b* for applying electrical current to an electrolyte within the cell, thereby generating a gas within the cell corresponding to the electrical current applied. The so-generated gas is applied to the gas chamber of its respective drug reservoir **50**, i.e., between a displaceable diaphragm **50***a* (FIG. **7**) and a rigid cover **50***b*, to thereby contract the drug reservoir and to feed its drug via its conduit **54** to the outlet cavity **56**, which is in communication with the injection needle **58**.

The electrolytic cells **52** are energized by a battery **62** (FIG. **7**) under the control of microprocessor **64** via electrical conductors **66** carried by a printed circuit board **68** connected to the electrodes **52***a*, **52***b* of each electrolytic cell.

It will be seen that including a plurality of drug reservoirs **50** each separately controllable by its own gas generator **52**, enables the device to be controlled to provide a wide range of dispensing rates. The series connections of the drug reservoirs with the outlet cavity **56**, which is in communication with the injection needle **58** permits the device to be conveniently primed by injecting the drug via injection plug **60** into all the reservoirs in series until the drug begins to discharge through the needle.

The Embodiment of FIG. **9**

FIG. **9** illustrates a variation in the construction of the device of FIGS. **7** and **8**, in that the plurality of drug reservoirs, therein designated **150**, are connected, via their respective conduits **154**, to the outlet cavity **156**, which is in communication with the injection needle **158**. As in the device of FIGS. **7** and **8**, the device of FIG. **9** is also provided with a separate gas generator **152**, e.g., an electrolytic cell, for each of the plurality of drug reservoirs **150**. Each reservoir is separately filled via its own injection plug **160**.

It will be seen that the device illustrated in FIG **9** permits the delivery of a single drug, or a mixture of drugs, all under the control of the microprocessor (e.g., **64**, FIG. **7**). Thus, if a large quantity of drug is to be delivered, the microprocessor could be preprogrammed to energize a plurality of the electrolytic cells **152** at one time; and if two or more drugs are to be simultaneously delivered, the various reservoirs **150** would be filled with the respective drugs and dispensed as required under the control of the microprocessor.

The Embodiments of FIGS. **10** and **11**

While in the above-described embodiments, the lower surface of the housing (e.g., **4**) is flat, FIGS. **10** and **11** illustrate variations in this construction. Thus, FIG. **10** illustrates the housing **102** having a housing section **102***a* of convex configuration on the lower surface **104** and coated with the pressure-sensitive adhesive **106**. A diaphragm **108** divides the interior of the housing into a drug reservoir **110** and a gas chamber **112** containing an electrolytic cell gas generator **116**. The hollow needle **114** extends through the center of the lower surface **104** of the housing, and is dimensioned as described above to penetrate through the epidermis of the subject's skin. FIG. **11** illustrates a similar construction, except that the housing section **202***a* of the housing **202** is formed with a central projection **202***c* through which the hollow needle **214** extends.

The constructions of FIGS. **10** and **11** counteract the natural resilience or stretching of the skin when the device is applied, so as to achieve penetration of the epidermis by the needle. The use of a narrow diameter hollow needle minimizes trauma, minimizes leakage, and better ensures more controlled delivery.

EXAMPLE 1

A device according to the instant invention containing 0.6 ml of a solution of insulin (100 I.U./ml) was affixed to each of two rabbits and the devices were switched on. The insulin solution was infused at a rate of 0.1 ml/hour for two hours. As shown in FIG. **12**, blood glucose concentrations for these rabbits were measured at various times following activation of the devices. At one hour, mean blood glucose concentration had fallen from a control value of 6.25 mmol/l to 3.2 mmol/l. This value stayed relatively constant at 1.5 hours following activation (2.65 mmol/l) and at 2 hours (2.5 mmol/l), at which time the devices were removed. One hour later the mean value was 3.7 mmol/l, which value continued to rise with time.

EXAMPLE 2

A device according to the instant invention containing 0.6 ml of a solution of salmon calcitonin (1.0 mg/ml) was affixed to each of four rabbits. This solution was infused at the rate of 0.1 ml/hour for 6 hours. Serum calcium concentrations were measured via an ear vein at 0, 0.5, 1, 2, 3, 4, 5, and 6 hours following activation of the device, at which point the device was removed. As shown in FIG. **13**, mean calcium concentrations fell steadily throughout the period of application and reached values representing 62.5% and 66.6% of the control values at 5 and 6 hours respectively.

While the invention has been described with respect to several preferred embodiments, it will be appreciated that these are set forth merely for purposes of example, and that many other variations, modifications and applications of the invention may be made.

What is claimed:

1. An intradermal drug delivery device for delivering a liquid drug to a subject via the subject's skin, comprising:

(a) a housing having a lower surface for application to the skin of the subject;

(b) means for affixing the housing in position with the lower surface in contact with the subject's skin;

(c) a plurality of drug reservoirs within the housing;

(d) an outlet cavity in communication with the plurality of drug reservoirs;

(e) a single hollow needle associated with the outlet cavity extending through the lower surface, having an inner end communicating with the outlet cavity and an outer end projecting outwards a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is pressed against the skin; and

(f) means for actively discharging the drug from the drug reservoir to the subject's skin via the needle; wherein the lower surface is shaped such that when it is pressed against the skin a substantial proportion of the pressure applied to the skin is directed through the outer end of the needle.

2. The device according to claim **1**, wherein all the drug reservoirs communicate in series with the outlet cavity.

3. The device according to claim **1**, wherein all the drug reservoirs communicate in parallel with the outlet cavity.

4. The device according to claim **1**, further comprising electrical means for individually controlling the feeding of drug from the plurality of reservoirs to the outlet cavity.

5. The device according to claim **1**, wherein the means for affixing the housing in position comprises a pressure-adhesive coating on the lower surface thereof.

6. The device according to claim **1**, wherein the lower surface of the housing is flat.

7. The device according to claim **1**, wherein the lower surface of the housing has a convex shape and the hollow needle extends from the center of the convexity.

8. The device according to claim **1**, wherein the lower surface of the housing is provided with a protuberance from which the needle projects.

9. The device according to claim **1**, wherein the lower surface of the housing is of a conical shape and the hollow needle extends from the apex of the cone.

10. The device according to claim **1**, wherein the needle projects outwards of the housing by 0.3–3.0 mm and has an outer diameter of 0.1–0.2 mm and an inner diameter of 0.05–0.075 mm.

11. The device according to claim **1**, wherein the needle projects outwards of the housing by 0.3–1.0 mm.

12. The device according to claim **1**, wherein the plurality of reservoirs are in the form of expansible-contractible chambers which are expanded when filled with the drug and which can be contracted to dispense the drug therefrom.

13. The device according to claim **1**, wherein each drug reservoir when filled has a volume from about 0.2 ml to 10.0 ml.

14. The device according to claim **1**, wherein each drug reservoir has a volume from about 0.5 ml to 3.0 ml.

15. The device according to claim **1**, wherein the means for actively discharging the drug comprises a plurality of electrically controlled gas generators within the housing for generating gas to separately contract the plurality drug reservoirs in order to discharge the drug therefrom.

16. The device according to claim **15**, wherein each gas generator is an electrolytic cell.

17. The device according to claim **15**, further comprising a start button which is depressible in order to energize at least one of the plurality of gas generators and thereby to start discharging the drug from the outlet cavity.

18. The device according to claim **15**, further comprising an electronic circuit for controlling the time and rate of gas generation in each of the plurality of gas generators, thereby controlling the discharge of the drug from the outlet cavity via the plurality of drug reservoirs.

19. The device according to claim **18**, wherein the electronic circuit comprises a microprocessor which is programmable with respect to the time and rate of gas generation.

20. The device according to claim **1**, wherein the housing further includes means for detecting a condition in the body of the subject and means for controlling the delivery of the drug in response thereto.

21. The device according to claim **20**, wherein the means for detecting is a temperature sensor for sensing the temperature of the subject.

22. The device according to claim **20** wherein the means for detecting is a pulse rate sensor for sensing the pulse rate of the subject.

23. The device according to claim **20** wherein the means for detecting is a blood glucose sensor for sensing the blood glucose level of the subject.

24. The device according to claim **20** wherein the means for detecting is a blood pressure sensor for sensing the blood pressure of the subject.

25. The device according to claim **20** wherein the means for detecting is a pH sensor for sensing the pH of a body fluid of the subject and controlling the delivery of the drug in response thereto.

26. The device according to claim **1**, further comprising at least one liquid drug contained in the plurality of drug reservoirs.

27. The device according to claim **26**, wherein the drug is selected from the group consisting of peptides, proteins, hormones, analgesics, anti-migraine agents, anti-coagulant agents, anti-emetic agents, cardiovascular agents, anti-hypertensive agents, narcotic antagonists, chelating agents, anti-anginal agents, chemotherapy agents, sedatives, anti-neoplastics, prostaglandins and antidiuretic agents.

28. The device according to claim **1**, where the housing further includes means for controlling the delivery of the drug.

29. The device according to claim **28**, wherein the means for controlling comprises a processor.

30. A method of delivering a biologically effective amount of at least one liquid drug intradermally to a subject, comprising the steps of:

(1) affixing the intradermal drug delivery device according to claim **1** to the skin of the subject such that the needle penetrates throught the epidermis and into the dermis, wherein the plurality of drug reservoirs contain a biologically effective amount of at least one liquid drug; and

(2) activating the means for actively discharging the at least one drug so as to deliver a biologically effective amount of the at least one drug to the subject.

31. The method according to claim **30**, wherein the drug is selected from the group consisting of peptides, proteins, hormones, analgesics, anti-migraine agents, anti-coagulant agents, anti-emetic agents, cardiovascular agents, anti-hypertensive agents, narcotic antagonists, chelating agents, anti-anginal agents, chemotherapy agents, sedatives, anti-neoplastics, prostaglandins and antidiuretic agents.

32. The method according to claim **30**, wherein at least two drugs are administered simultaneously or sequentially.

* * * * *

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United States Patent [19]

Gross et al.

[11] Patent Number: 5,807,375

[45] Date of Patent: Sep. 15, 1998

[54] **ANALYTE-CONTROLLED LIQUID DELIVERY DEVICE AND ANALYTE MONITOR**

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[73] Assignee: **Elan Medical Technologies Limited**, Athlone, Ireland

[21] Appl. No.: **556,744**

[22] Filed: **Nov. 2, 1995**

[30] **Foreign Application Priority Data**

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[52] **U.S. Cl.** **604/890.1**; 604/66; 128/632; 128/635

[58] **Field of Search** ..... 604/65, 66, 67, 604/131, 132, 133, 890.1, 892.1; 128/632, 635, 642, DIG. 12, DIG. 13

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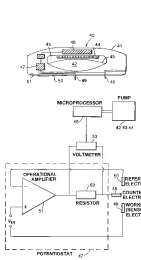
Primary Examiner—Mark Bockelman
Assistant Examiner—A. T. Nguyen
Attorney, Agent, or Firm—Kathleen L. Maher

[57] **ABSTRACT**

A liquid delivery device comprising a housing having a lower surface for application to the skin of a subject and having a reservoir and a gas generation chamber therein separated by a displaceable membrane. Gas generated by an electrolytic cell under the control of a microprocessor causes the gas generation chamber to expand and the reservoir to contract, thereby discharging a liquid drug, such as insulin, from the reservoir via a hollow delivery needle extending from the lower surface. The delivery needle and a sensor needle both extend from the lower surface a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is pressed against the skin. The sensor needle has an enzymatic coating for the detection of an analyte, such as glucose in the subject's plasma. The delivery needle is made of platinum-iridium, and a current passes between the needles and a potentiostat circuit according to the amount of glucose detected. A reference electrode (silver/silver chloride) which rests against the subject's skin increases the accuracy of the glucose measurement. The current through the potentiostat circuit is measured by a voltmeter and a signal from the voltmeter is amplified and communicated to the microprocessor which determines the correct rate of delivery of the drug on the basis of the level of analyte detected in the subject's plasma.

22 Claims, 10 Drawing Sheets



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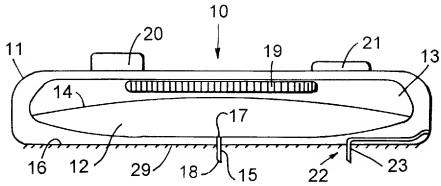
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FIG. 1

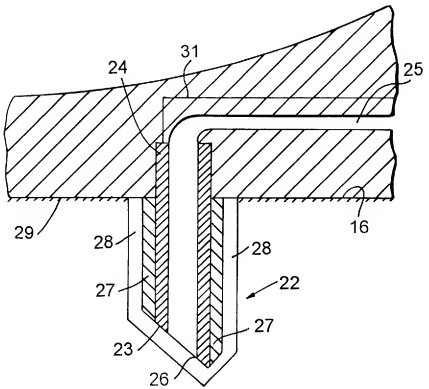
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FIG. 2

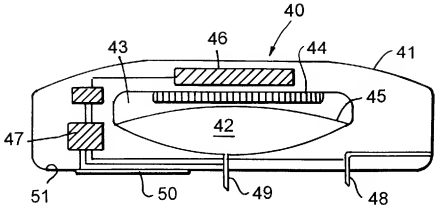

FIG. 3

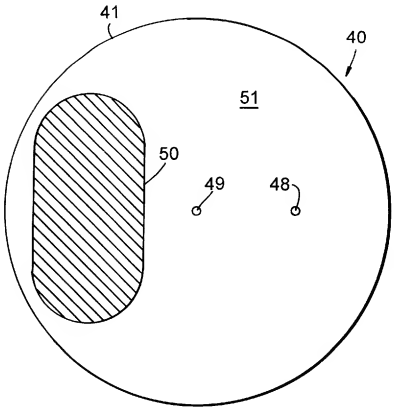

FIG. 4

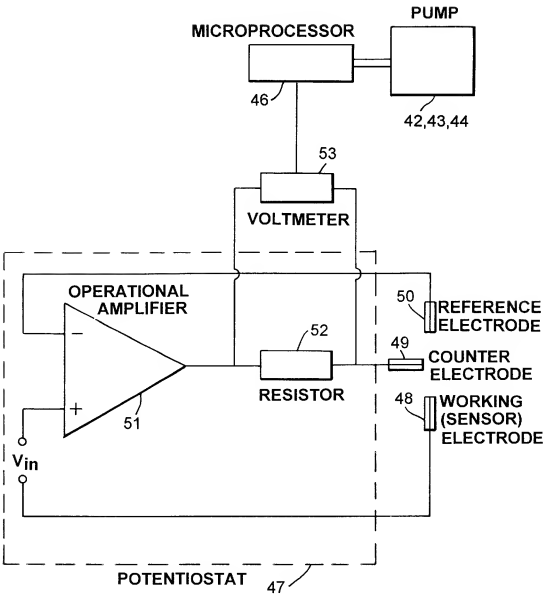
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FIG. 5

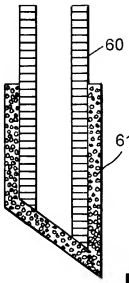

FIG. 6

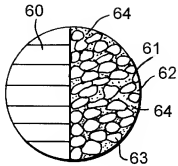

FIG. 7

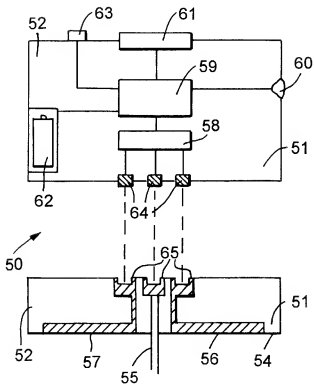

FIG. 8

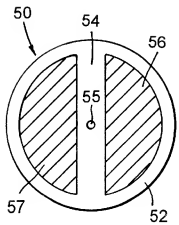

FIG. 9

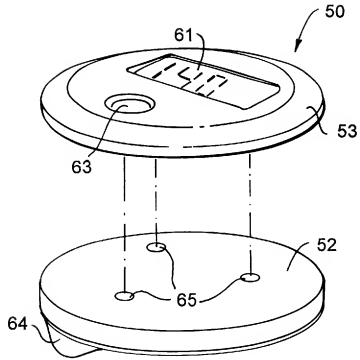

FIG. 10

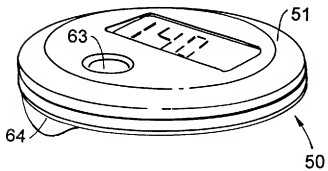

FIG. 11

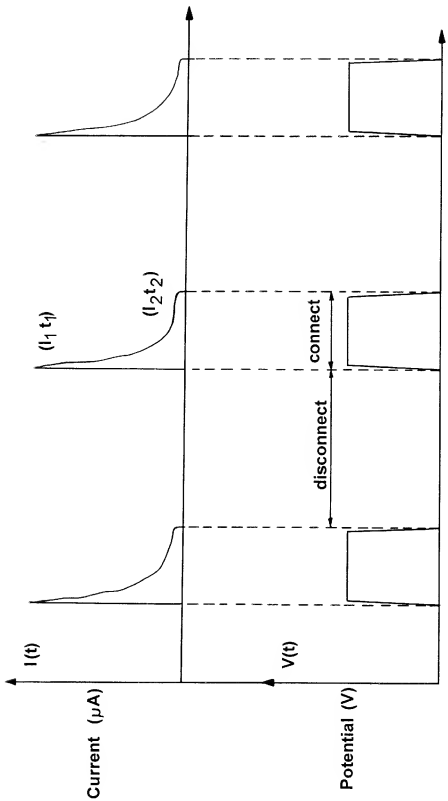
I(t)
V(t)
Current (μA)
Potential (V)
Time (ms)
(I1 t1)
(I2 t2)
connect
disconnect

FIG. 12

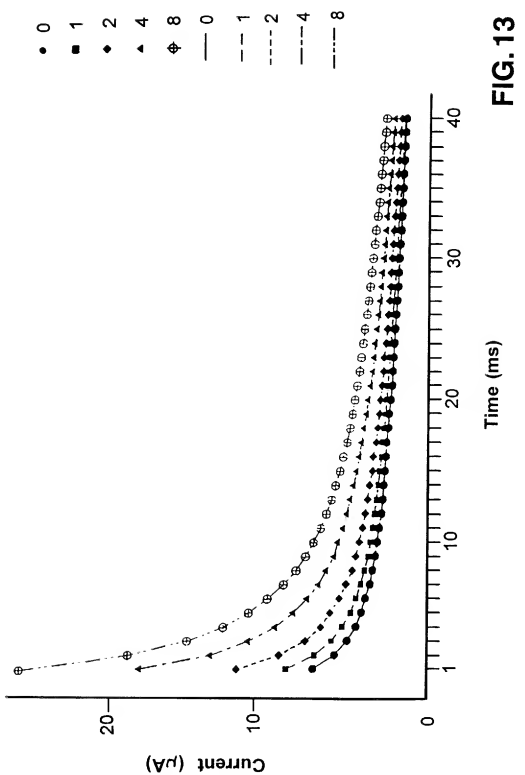

FIG. 13

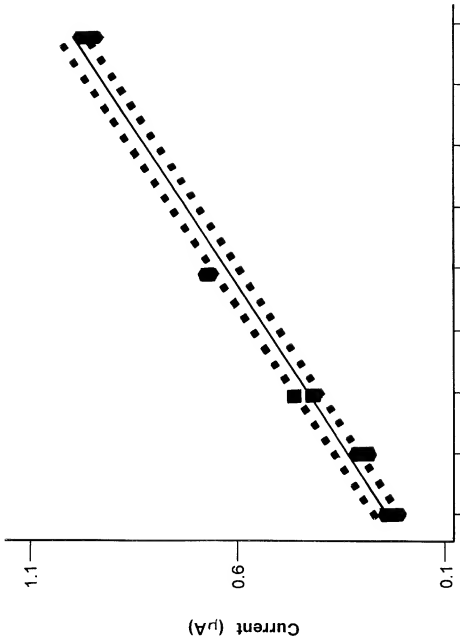

FIG. 14

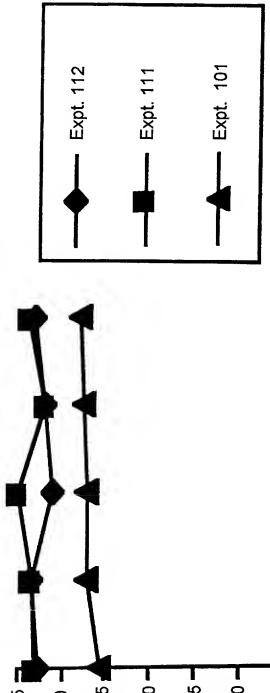
Expt. 112
Expt. 111
Expt. 101
I(t1)/I(t2)
35
30
25
20
15
10
5
0
0
1
2
4
8
Glucose Conc. (mM)

FIG. 15

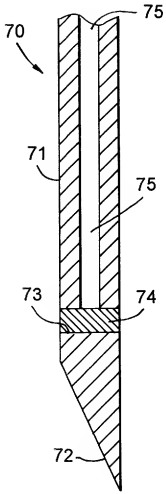

FIG. 16

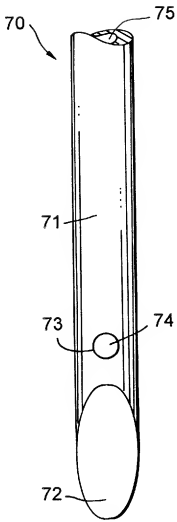

FIG. 17

ANALYTE-CONTROLLED LIQUID DELIVERY DEVICE AND ANALYTE MONITOR

FIELD OF THE INVENTION

This invention relates to devices for the delivery of liquid drugs to a subject via the subject's skin, and in particular to "closed loop" insulin delivery devices, as well as to analyte sensors for use in "closed loop" and "open loop" delivery systems.

BACKGROUND OF THE INVENTION

Conventional therapy for insulin-dependent diabetes mellitus involves self-administered subcutaneous insulin injections a number of times daily (usually two, three or four times). The dosage regime is designed to maintain the blood glucose level (glycemia) of the subject between hypoglycemic and hyperglycemic levels, preferably between 3 and 10 mmol/l, taking into account variations arising as a result of, for example, glucose intake at mealtimes and glucose elimination during periods of activity.

In order to provide better control of a subject's glycemia, continuous infusion pumps have been developed to deliver glucose at a basal rate. This rate may be pre-programmed, or the patient or physician may manually control the rate according to the results of successive blood glucose tests (which can be carried out by the patient using apparatus which provides a result within a matter of minutes). The basal rate is usually supplemented by bolus injections before meal times. Such pumps are known as "open loop" systems.

A subcutaneous catheter is used to deliver insulin from an infusion pump to the patient. The open wound caused by the catheter means that the catheter must be resited every few days. Complications arising from the use of the catheter can include erythemia, abscesses, cellulitus and, occasionally, systemic infection.

Implantable devices are also known. Such devices are generally implanted in the abdomen. Complications arising from the use of implantable devices include infection, particularly of the implantation site, and skin necrosis over the implant.

"Closed loop" systems comprise an insulin pump controlled by a microprocessor and a glucose sensor linked to the microprocessor. The rate or frequency of insulin administration is controlled by the microprocessor according to the instantaneous blood glucose level measured by the sensor. Because a system of feedback similar to natural homeostatic regulation is used, a closed loop insulin delivery system may also be referred to as an "artificial pancreas".

In general, closed loop systems are not implanted. Many of the known systems are of the so-called bedside type which include a reservoir and a pump for a hypoglycemic agent (such as insulin), a reservoir and pump for a hyperglycemic agent (such as glucagon or glucose), means for injecting each agent into the body, means for measuring the blood glucose levels, means for controlling the delivery of each agent at a rate determined by the measured blood glucose level and a housing containing the reservoirs, pumps, measuring apparatus and controlling means. The size of this type of artificial pancreas means that it is limited to bedside use (which explains the name). Furthermore, because the means for measuring blood glucose levels requires the collection of blood from the patient, this mode of therapy imposes a heavy burden on the patient, so that it is impossible to use the device continuously for a long period of time.

A portable artificial pancreas is known from EP-A-0 098 592. The artificial pancreas has a reservoir for a blood sugar control agent and a feed pump adapted to inject the control agent into the subject's body at a rate determined by a microcomputer. The microcomputer receives a signal from a glucose sensor which is inserted into the subject's body, and calculates the required insulin delivery rate from the detected glucose level. The glucose sensor and the injection unit (which includes the reservoir, the pump and the microcomputer), are separate from one another and the output signal of the sensor is transmitted to the microcomputer by radio.

In the preferred embodiment, a detection unit, including the sensor and radio transmitter, is in the form of a wristwatch having a tube leading therefrom to a catheter which has the blood glucose sensor at the end thereof. The injection unit, which includes a radio receiver for receiving the signal from the detection unit, is adapted to be worn on a belt.

This type of portable artificial pancreas shares the problems associated with open loop systems (i.e. erythemia, abscesses, cellulitus and systemic infection), but the problems are, in fact, magnified because two catheters are used instead of one.

Apart from the strictly medical problems associated with existing pumps, a significant amount of pain and trauma is also associated with the application of known devices when the catheter(s) is/are inserted into the skin.

Furthermore, such devices are inconvenient to use and may cause discomfort as the pumps are often quite bulky and are generally worn on a belt or a shoulder strap, as is the case with the injection unit of EP-A-0 098 592.

Although implantable devices have found a limited success in open loop systems, they are unsuitable for use in closed loop systems as a failure of the sensor pump, or controlling equipment, or the blockage of an outlet (which might occur as a result of a build-up of fibrin, for example), can lead to ketoacidosis. A patient using an open loop system will be supplementing the basal rate with bolus injections and may be carrying out regular blood glucose tests as before. According, there is far less danger of severe hypoglycemia or hyperglycemia occurring if an implanted open loop system fails than would be the case for a patient with an implanted closed loop system.

Portable closed loop systems, such as the system described in EP-A-0 098 592, require a reliable glucose sensor. The sensor employed in EP-A-0 098 592 comprises a platinum electrode and a silver electrode. The platinum electrode and silver electrode form part of an electric circuit in which hydrogen peroxide is electrolysed. The hydrogen peroxide is produced as a result of the oxidation of glucose on a glucose oxidase membrane, and the current through the circuit provides a measure of the hydrogen peroxide concentration, and hence the glucose concentration, in the vicinity of the sensor.

The sensor is in the form of a composite electrode comprising both the platinum and silver electrodes, a glucose oxidase membrane layer, a polyurethane film which is permeable to glucose, oxygen and hydrogen peroxide, and a steel, glass and plastics supporting structure. The composite electrode is attached to the forward end of the catheter which is inserted into a blood vessel or beneath the skin of the subject.

The accuracy of the electrode (and accordingly, the accuracy of the controlled delivery of insulin or glucagon) depends on the efficient conversion of glucose and oxygen to give gluconic acid and hydrogen peroxide. The amount of

hydrogen peroxide must be reliably linked to the amount of available glucose in the bloodstream. False determinations may, however, arise with the sensor described in EP-A-0 098 592 because all of the available glucose may not be converted by the glucose oxidase enzyme if there is an insufficient supply of oxygen.

Oxygen is available in dissolved form in the blood and it occurs as a product of the electrolysis of hydrogen peroxide. However, the assumption that excess oxygen will be available relative to glucose may not be correct. If oxygen is not available in excess, then the amount of available oxygen (not glucose) will be the limiting factor in the reaction and the current provided by the electrode will provide a false determination of the subject's glycemia.

The ultimate intention of manufacturers of closed loop systems is to devise a system which provides the subject's entire insulin requirement without there being any need for self-injection of bolus insulin. Accordingly, any such system must be acceptable to the patient in terms of being as unobtrusive as possible, being minimally painful and traumatic in application and use, providing minimum discomfort during administration, as well as being of the utmost reliability and efficiency. These objectives are not met by the devices of the prior art, for the reasons outlined above, and it is an object of the present invention to provide a device having the above-mentioned qualities.

A further aspect of the invention relates to a sensor per se for use in conjunction with an open loop system, to provide an indication that the rate of drug delivery should be varied or that a bolus injection should be administered. It can also be used in conventional diabetes therapy to replace the uncomfortable and potentially unreliable and dangerous method of self-administered blood tests at various intervals throughout the day.

One of the primary problems associated with conventional diabetes therapy (i.e. self-injection of insulin, optionally preceded by a blood test) is its susceptibility to human error. A diabetic whose blood level has become unexpectedly hypoglycemic, e.g. as a result of unforeseen or unexpectedly strenuous activity or as a result of prolonged abstinence from sugar-rich nourishment, is in severe danger of entering a hypoglycemic coma. The danger is compounded by the fact that the time lost between onset of hypoglycemic symptoms and an actual comatose state can be very short, and by the fact that hypoglycemia has a profound psychological effect which is superficially similar to drunkenness in that the patient becomes giddy and loses inhibitions and a sense of responsibility. Furthermore, uninformed bystanders may in fact mistake hypoglycemic symptoms for drunkenness.

Bearing the above factors in mind, it would be desirable to provide means by which a patient can ascertain his or her blood glucose level as desired without the inconvenience of obtaining a blood sample and carrying out a blood glucose test.

Another object of this aspect of the invention is the provision of a blood glucose monitor which informs the diabetic (and, optionally, people in the vicinity) that the blood glucose levels are abnormally low or high, as the case may be, thereby allowing the diabetic to take corrective action, such as the intake of a sugar-rich drink, for example or an injection of insulin, depending on whether hypoglycemia or hyperglycemia is indicated.

Bearing in mind that relatively sophisticated and/or costly electronic circuits may be used in such a monitoring device, it is highly desirable to minimise the expense involved in manufacturing the device. This is particularly true in the case of a device employing an enzymatic sensor, since such a sensor will probably have quite a short life span necessitating frequent replacement. Even a significantly advantageous invention, improvement or modification will not achieve its commercial potential if, in the opinion of the consumer the expense is not justified by the advantages.

A further problem associated with enzymatic sensors which are intended for use by patients under real life conditions, as opposed to experimental prototypes, is that of sensor degradation. Even if a sensor is calibrated, it can become damaged, inefficient or inaccurate as a result of incorrect application, abrasion, manufacturing flaws, changes in enzyme activity with time or changes in the transport properties of protective membranes surrounding the sensor due to interactions with foreign materials.

A paper by Rishpon J. (Biotechnology and Bioengineering, Vol. XXIX, pages 204–214 (1987)) deals with improved glucose oxidase enzyme electrodes and provides a method of determining some of the parameters affecting electrode efficiency from the signal obtained. The experiment described uses platinum disc electrodes covered by a glucose oxidase enzyme layer cross-linked to bovine serum albumen. The electrode is initially held for 10 seconds at 0.0 volts and then stepped to 0.8 volts for 10 seconds. This square wave potential pattern is repeated and the current is measured. The current is digitized and fed to a microcomputer every 200 μs. These individual current readings were averaged to provide improved resolution, but were nevertheless found to give unsatisfactory resolution and signal to noise ratio. Accordingly, the current readings were integrated to provide coulometric rather than amperometric data. This coulometric data was then analysed to provide kinetic and transport parameters relating to the electrodes and it was found that the analysed data could be used in the evaluation of various electrode types.

The present invention seeks to provide a determination of sensor quality or degradation when the sensor is used in vivo on an on-going basis without requiring extensive computations and analysis, and providing direct results rather than abstract parameters such as diffusion coefficients (as obtained by Rishpon).

Yet a further object of the invention is to provide improved signal to noise ratios using direct measurements, without requiring complex multiple measurements, averagings and integrations. In this respect, it should be noted that the background noise in measuring glucose activity may be greatly increased by the presence of materials such as paracetamol which interfere with the accuracy of glucose measurements by the enzymatic sensor. In the amperometric measurements described by Rishpon, unsatisfactory resolution and signal-to-noise ratios were obtained before integration was effected, and it should be noted that each data point on the amperometric graph described by Rishpon as "unsatisfactory" in fact represented the averaging of 2500 distinct measurements.

SUMMARY OF THE INVENTION

Accordingly, the invention, in a first aspect, provides a liquid delivery device for delivering a liquid drug to a subject via the subject's skin at a rate sufficient to maintain plasma levels of an analyte within a physiologically acceptable range, comprising:

a housing having a lower surface for application to the skin of the subject;

means for holding the housing in position with the lower surface against the subject's skin;

a drug reservoir within the housing;

a hollow delivery needle associated with the drug reservoir extending through the lower surface when the lower surface is in contact with the subject's skin, having an inner end communicating with the drug reservoir and an outer end projecting outwards a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is pressed against the skin;

means for actively discharging the drug from the reservoir to the subject's skin via the needle;

means for detecting the concentration of an analyte in the subject's plasma and for providing an electrical signal in accordance with the detected concentration, the concentration of said analyte being directly or indirectly related to the amount of drug required by the subject; and

means for receiving said electrical signal and for controlling the rate of active discharge of drug in response thereto.

The term "liquid" as used herein includes pure liquids, solutions, suspensions, low-viscosity gels and other flowable compositions. The term "drug" includes pharmaceutical, therapeutic, diagnostic and nutritional agents, and compositions containing such agents.

The device according to the invention is far less painful in application and use if suitable needle dimensions are chosen. Preferably, the needle is of a suitable length to penetrate the patient's skin either intradermally (i.e. the tip of the needle extends to a point within the dermis) or subcutaneously (the tip of the needle penetrates through the dermis into the underlying tissue).

The device can be pressed against the skin and this action ensures correct insertion of the needle. If a narrow needle, preferably having an outer diameter of less than 0.2 mm, is used, only the minimum amount of trauma will be associated with the application of the device.

Furthermore, as the manner of insertion of the needle is invariable (the device is pressed against the skin and the needle always penetrates the skin correctly), the subject can personally apply the device without having to take any particular precautions or without having to receive any medical training. This is not the case with the devices of the prior art, which require, for example, catheters to be inserted intravenously or subcutaneously. In conventional insulin therapy, the patient must be taught to administer subcutaneous injections, and if sufficient care is not taken the injection may be intravenous or intramuscular rather than strictly subcutaneous, or the needle may hit a bone under the skin. If the injection is delivered to the wrong environment (vein or muscle), the uptake of drug will not occur at the correct rate. The risks associated with these occurrences are significant drawbacks to known systems.

For the above reasons, the invention provides a significant advantage over known closed loop systems, making it suitable for unsupervised use. As the device also has means for holding the housing in position with the lower surface against the subject's skin, the device is completely portable and may be worn inconspicuously on the body under all clothing without requiring a belt or a bracelet-type strap.

Furthermore, as the device is not a two-part system, as is the case with EP-A-0 098 592, the signal may be communicated directly from the means for detecting the blood concentration of an analyte to the means for controlling the rate of active discharge of the drug. Accordingly, there is no danger of the signal from the sensor being misinterpreted due to radio interference from nearby sources, and the device itself cannot interfere with nearby equipment.

The device is also less expensive to manufacture than the relatively complex portable artificial pancreases of the prior art. It can be disposable and is preferably designed for once-daily administration. Suitably, the device is applied in the morning and worn throughout the day. It may be removed at night or worn throughout the night. If removed, the subject may inject a conventional night-time dose of insulin or the device may be adapted to deliver a suitable bolus of insulin before removal.

Suitably, the delivery needle extends permanently through the lower surface.

Preferably, however, said delivery needle is recessed within the housing when the lower surface is not in contact with the subject's skin, and the device comprises means for extending the delivery needle through the lower surface so as to project outwards said distance when the housing is pressed against the skin. This may be achieved, for example, by means of a mechanical, electrical or piezoelectric sensor located on the lower surface of the housing, with the sensor means for extending the delivery needle through the lower surface being actuated by the sensor. The extension of the delivery needle is carried out in a consistent and suitable manner when this embodiment is used.

Preferably, the delivery needle penetrates through the dermis for subcutaneous delivery of the drug. The choice of intradermal or subcutaneous delivery, however, depends on the condition to be treated, the drug to be used and the chosen therapy and dosage regime. For certain drugs, it is preferable to deliver dosages intradermally as a depot effect may be desired, i.e. the drug builds up in concentration within the skin layers and is gradually released therefrom to the systemic circulation. With suitable drugs this depot effect can provide therapeutically effective blood levels many hours after the device has been removed.

According to a further embodiment of the invention, the means for detecting the plasma concentration of the analyte comprises a sensor needle extending from the lower surface of the housing when the lower surface is in contact with the subject's skin, the sensor needle having an outer end projecting outwards a sufficient distance so as to penetrate through the epidermis and into the dermis when the housing is pressed against the skin.

Thus, the application of the housing can ensure the insertion of both the delivery needle and the sensor needle for the analyte. This is particularly advantageous for the reasons recited above in relation to the delivery needle.

Suitably, the sensor needle extends permanently through the lower surface.

Preferably, the sensor needle is recessed within the housing when said lower surface is not in contact with the subject's skin, and the device comprises means for extending the sensor needle through the lower surface so as to project outwards said distance when the housing is pressed against the skin.

The same means may be used to extend both the delivery needle and the sensor needle simultaneously through the lower surface when the device is pressed against the skin. Alternatively, each needle may be activated separately as the particular parts of the housing adjacent to the point through which the needles extend comes into contact with the skin.

Suitably, the sensor needle penetrates through the dermis. It is envisaged that the same needle may be used for the purposes of delivery and analyte sensing. Preferably, however, the delivery and sensor needles are in spaced apart relationship.

In a preferred embodiment, the delivery and sensor needles are electrically conducting and the means for detect-

ing the concentration of an analyte is to measure an electric current between the needles, the circuit being completed upon application of the lower surface to the skin of the subject. The third reference voltage point kept at a specific voltage compared to the sensor needle an electric circuit comprising a power source connected between the needles, the needles may be entirely formed of conductive material or they may carry a conductive coating or conductive elements therein.

Suitably, the electrical signal provides a measure of the electric current flowing through the circuit.

Suitably, the sensor needle has an enzyme associated therewith, the enzyme being specific to the analyte to be detected and the current through the circuit being dependent on the concentration of a reactant in the enzymatic reaction in the vicinity of the needle.

Preferably, the sensor needle has an enzyme associated therewith, the enzyme being specific to the analyte to be detected and the current through the circuit being dependent on the concentration of a product of the enzymatic reaction in the vicinity of the needle.

The use of an analyte-specific enzyme is particularly advantageous as such an enzyme can be used to detect minute concentrations of analyte in the blood, plasma or tissue of the subject. The association of an electric current with the enzymatic reaction allows a quantitative evaluation of analyte concentration. The electrical current may, of course, be amplified or analysed as appropriate by means of any one of a vast range of electronic techniques. Furthermore, the enzyme allows high concentrations of analyte to be measured equally accurately as only a very small quantity of enzyme can catalyse large amounts of substrate (analyte). Some pure enzymes, for example, can catalyse the transformation of as many as 10,000 to 1,000, 000 mols of substrate per minute per mol of enzyme. Accordingly, only a very small enzyme supply needs to be associated with the needle to ensure total analyte reaction in the vicinity of the needle.

Preferably, the product of the enzymatic reaction is a charged species, or said product spontaneously breaks down to produce a charged species, or said product reacts catalytically at the surface of the needle to produce a charged species. The term "charged species" as used herein includes ions, protons and electrons. In any of these situations, the production of charged species in the vicinity of the needle allows a current to flow between the electrodes. Accordingly, the current through the circuit is dependent on the numbers of charged species available to carry current at any time.

Suitably, the product of the enzymatic reaction or a derivative thereof partakes in an electrochemical reaction, the sensor needle acting as one electrode of an electrochemical cell and the delivery needle acting as another electrode. In accordance with Faraday's Laws of Electrolysis, the amount of a substance consumed at an electrode of an electrochemical cell is directly proportional to the current through the cell. Obviously, one would not expect this strict relationship to hold for an electrochemical cell incorporating a complex biological system, but the circuit can nevertheless be calibrated to provide a correlation between the current and the analyte concentration.

Suitably, when the enzymatic reaction requires free oxygen to proceed, the structure of the sensor needle allows oxygen to pass from an inner end thereof which is in communication with a supply of oxygen to the exterior surface of that part of the sensor needle which projects from the housing.

Preferably, the needle is a hollow needle open at the outer (skin-penetrating) end to provide communication between the inner end and the enzyme.

The use of a hollow needle (or of some other structure of needle which allows oxygen to reach the location of the enzyme) confers an important advantage over conventional implanted enzyme sensors, as the hollow needle ensures that the rate of reaction is never restricted by a lack of oxygen. The supply of oxygen may be air inside the housing, air outside the housing, an oxygen reservoir within the housing or an oxygen source (such as an electrochemical cell) inside the housing, to provide a few examples.

Preferably, the enzyme is in the form of an enzyme-containing coating on the surface of the needle.

Further, preferably, the enzyme-containing coating is covered by a protective coating of an analyte-permeable material.

Suitably, said analyte-permeable material is a perflourinated ion-exchange membrane, for example, "Nafion" ("Nafion" is a Trade Mark). This type of material protects the enzyme before and during operation of the device. If the sensor is in the form of a hollow needle, the coating may cover the open end of the needle to prevent fluids from entering the needle.

According to a preferred embodiment, the analyte is glucose, and the drug is selected from glucagon and insulin or analogues thereof.

The insulin used in the device may be chosen to meet the requirements of the patient. It may be bovine, porcine, human or synthetic and it may be short acting or long acting, or it may comprise a mixture of different types of insulin.

Preferably, in this preferred embodiment of the invention, the enzyme is glucose oxidase.

Further, preferably, the product is hydrogen peroxide.

In the preferred embodiment, the hydrogen peroxide is catalysed to produce oxygen, hydrogen ions and electrons and the magnitude of the current through the circuit is related to the number of electrons produced.

Suitably, the hydrogen peroxide is produced adjacent to a platinum supply, the platinum supply catalysing the oxidation of the hydrogen peroxide. The platinum may be in a colloidal dispersion within a coating on the surface of the sensor needle, it may be carried by particles distributed in intimate admixture with the enzyme supply, it may be provided on the surface of the sensor needle, or the sensor needle may comprise platinum or a platinum alloy such as platinum-iridium.

A high degree of accuracy may be achieved if the electric circuit comprises a reference electrode which is adapted to contact the subject's skin and the sensor needle is biased at a fixed potential with respect to the reference electrode.

Suitably, the electric circuit comprises a potentiostat having an operational amplifier which drives a current between the sensor and delivery needles.

Further, preferably, the power source and the sensor needle are connected in series with the positive input of the amplifier, and a resistor and the delivery needle are connected in series with the amplifier output, the reference electrode being connected to the negative input of the amplifier.

As will be further described below, the potentiostat maintains the potential of the sensor needle at a preset level with respect to the reference electrode by passing the current between the sensor needle and the delivery needle. Thus, the sensor needle acts as a working electrode and the delivery needle acts as a counter electrode.

The current through the reference electrode is, in a well calibrated potentiostat, minimal and the current between the

working electrode and the counter electrode is independent of the resistance in the "cell" (in this case the skin and tissue between the needles). Thus, the current is limited by the numbers of mobile charged species available to carry current.

Suitably, the current through the circuit is determined by measuring the voltage drop across the resistor.

Preferably, a voltmeter connected across said resistor provides a signal determined by the magnitude of the voltage drop and the signal is amplified and supplied to the means for receiving an electrical signal and for controlling the rate of active discharge of drug in response thereto.

Further, preferably, said means for controlling the rate of active discharge is a pre-programmable microprocessor which calculates the required drug dosage from the received signal and which controls the rate of active discharge in order to provide the required dosage.

Optionally, the circuit comprises switching means to allow current to flow intermittently. In this embodiment, the time taken for the current to reach a steady state (if a steady state is reached) can be analysed to determine information regarding the operation of the device. Suitably, therefore, a charge accumulates at the sensor needle when current is prohibited and the charge disperses when current flow begins.

An explanation of how a pulsatile current can be used to derive useful information on transport and kinetic parameters is given in the paper by J. Rishpon referred to above. By using a voltage stepped periodically between 0.0V (10 seconds) and 0.8V (10 seconds) and observing the resultant current specifically by sampling the current at intervals of 200 μs and integrating the digitized signal to obtain a chronocoulometric response, sensitivity was greatly increased above that available by steady-state measurements. However, sophisticated equipment including a microcomputer was required to digitize, average and integrate the current measurements.

Preferably, the switching means comprises means for intermittently applying a voltage to the sensor needle. Suitably, the voltage is applied as a stepped voltage. As the enzymatic reaction proceeds independently of the current, a charge will accumulate at the sensor needle when the current is switched off. When the current is switched on, the charge is able to disperse, and the current takes the form of a peak which falls away to a steady state level.

Preferably, the current is measured immediately after the stepped voltage is applied. This enables a large current to be measured and improves the signal to noise ratio.

In a preferred embodiment, the circuit further comprises means for comparing the current at different times. This information can be used to evaluate the efficiency and condition of the electrode. In one embodiment, the current is measured twice at times t_1 and t_2 as it falls from a peak level towards a steady state level, given value $I(t_1)$ and $I(t_2)$. The ratio $I(t_1)/I(t_2)$ has been found to be a constant which is specific to the electrode and which is independent of the concentration of the analyte being measured. It is also been found that for any given construction of electrode, the ratio will remain constant as long as the electrode is functioning correctly, but when the ability to detect glucose is impaired, the ratio will change. Therefore, repeated measurements of this ratio provide a way of monitoring the quality of the sensor over time and the user can thereby be alerted when the sensor requires replacement.

To facilitate the application of the device, in a preferred embodiment, the lower surface is shaped such that when it is pressed against the skin a substantial proportion of the pressure applied to the skin is directed through the needle tip. Thus, the needle may project permanently from a suitable part of the lower surface or it may be extended from a suitable part of the lower surface when the lower surface is pressed against the skin. Preferably, the shape of the lower surface is adapted to compensate for the elasticity of the skin by the design of the lower surface. Generally, this means that the lower surface is shaped such that a substantial portion of the pressure is directed through the tip of the needle itself rather than through the skin-contacting parts of the lower surface, at least while the housing is being pressed against the skin.

Suitably, for example, the lower surface of the housing may have a convex shape and the hollow needle may extend from the centre of the convexity, or the lower surface may be provided with a protuberance from which the needle projects, or the lower surface may be of a conical shape with the needle extending from the apex of the cone (suitably, this is an inverted cone with a large base-to-height ratio).

Preferably, the means for affixing the housing in position comprises a pressure-adhesive coating on the lower surface thereof. This allows the device to be far less obtrusive than the sort of device which must be worn on a belt, shoulder strap or bracelet.

Suitably, the delivery and/or sensor needle(s) project inwards of the housing by 0.3–3.0 mm and have an outer diameter of 0.05–0.4 mm, preferably 0.1–0.3 mm, and an inner diameter of 0.02–0.1 mm, preferably 0.05–0.075 mm. Such needle dimensions allow for intradermal or subcutaneous delivery and a small outer diameter ensures that the application of the needle(s) is relatively painless.

In a preferred embodiment of the invention the reservoir is in the form of an expansible-contractible chamber which is expanded when filled with the drug and which can be contracted to dispense the drug therefrom. Suitably, the drug reservoir, when filled, has a volume of the order of 0.2 ml to 10.0 ml.

Further, preferably, the means for actively discharging the drug comprises an electrically controlled gas generator within the housing for generating a gas to contract the drug reservoir in order to discharge the drug therefrom. Suitably, the gas generator is an electrolytic cell. The use of an electrolytic cell is preferred as the generation of gas is highly controllable and is suitable for delivering accurate amounts of the drug, as well as for allowing the delivery of drug to be started and stopped substantially instantaneously if pulsatile delivery is required.

As a preferred feature, the device comprises a start button which is depressible in order to energize the gas generator and thereby to start discharging the drug from the drug reservoir.

Suitably, the means for controlling the rate of active discharge comprises an electronic circuit for controlling the time and rate of gas generation, thereby controlling the discharge of the drug from the drug reservoir.

Optionally, the device further comprises a membrane which is permeable to the drug and impermeable to solid impurities, the membrane covering the inner end of the delivery needle.

The invention provides, in a second aspect, a device for monitoring the concentration of an analyte in the plasma of a subject, comprising:

a housing having a lower surface for application to the skin of the subject;

means for holding the housing in position with the lower surface against the subject's skin;

an electrical detection circuit comprising a power source connected across two electrodes mounted on said lower

surface, the circuit being completed upon application of the lower surface to the skin of the subject, one of said electrodes being a sensor needle for penetrating through the epidermis and into the dermis when the lower surface is applied to the skin and having an enzyme associated therewith, said enzyme being specific to the analyte to be detected, and the current through the circuit being directly or indirectly dependent on the concentration of the analyte in the vicinity of the sensor needle; and

a communication circuit comprising means for measuring the current through said electrical detection circuit, means for calculating the plasma concentration of the analyte from the measured current and communicating means for communicating the calculated concentration to the subject.

The application of such a device is no more painful, and may, in fact, be less painful, than a conventional pin prick blood test. Unlike such a blood test, however, the device according to the invention need not be repeatedly administered if the blood levels need to be rechecked. The device may, in fact, be worn in place for continual monitoring over a period of, for example, 12 hours, one day, two days or up to one week. The period is generally limited by the exhaustion of, or a decrease in the efficiency of, the enzyme associated with the sensor needle. The presently preferred frequency of administration is once-daily as this ensures that the sensor needle is always in optimum condition and it also allows the subject to change the site of application regularly.

Suitably, the enzyme is glucose oxidase and the analyte to be measured is glucose.

The invention is not, however, limited solely to glucose monitoring devices. Similar enzymatic sensors may suitably be employed if alternative analytes require monitoring.

According to a preferred embodiment, the sensor needle is a working electrode and the other of said two electrodes is a counter electrode in the form of a platinum surface for contact with the subject's skin.

Although the counter electrode can be an invasive electrode (i.e. a needle) there is no necessity in the present case for a second needle, and in the interests of comfort, it is preferred to employ a counter electrode which rests against the skin. Preferably, the area of such an electrode is maximised to increase sensitivity. In certain cases, the sensitivity of an electrode resting against the skin may not be sufficient, and, accordingly, an invasive needle may be used.

Preferably, the electrical detection circuit also comprises a reference electrode on the lower surface of the housing, in the form of a silver/silver chloride surface for contact with the subject's skin, and a potentiostat having an operational amplifier which drives a current between the working electrode and the counter electrode.

Such a circuit operates as hereinbefore described with reference to the embodiments of the invention in its first aspect.

According to a particularly preferred embodiment, the housing comprises a first part and a second part, the first part comprising the lower surface and the electrodes and the second part comprising the power source and the communication circuit.

Suitably, the first part is detachably mounted on the second part, such that the first part can be disposed of and replaced and the second part can be reused a number of times.

When a two-part device is used, the costs can be considerably lower. The first part contains all of the disposable elements (adhesive, electrode coatings, etc.), while the second part contains the reusable elements, such as the electronic components, the communicating means and the power source. Although a power source such as a battery must be replaced periodically, it is a relatively permanent element in comparison to an enzymatic sensor. Long-term batteries can be used having a life span of over two years. Accordingly, such batteries can be reused hundreds of times relative to the first part.

Suitably, the communicating means is activated when the calculated analyte plasma concentration falls outside a predetermined range.

Further, suitably, the communicating means comprises an audible alarm.

Thus, an audible alarm can be made to sound if the subject has blood levels approaching those associated with hyperglycemia or hypoglycemia, and corrective action can be taken before any serious condition develops. Preferably, different sounds are emitted by the alarm depending on the condition of the patient. Furthermore, different sounds or louder sounds can be emitted if the situation worsens.

Preferably, the communicating means operates continuously to provide a constant indication of the subject's analyte plasma concentration.

Further, preferably, the communicating means comprises a visible display of the analyte concentration. Suitably, the visible display is in the form of a liquid crystal display for indicating the analyte concentration as a numerical value.

Other visible displays are, of course, possible, such as a series of light, with a number of lights lit indicating an approximate blood glucose level, or a dial indicating a numerical value relating to the blood glucose level, etc.

One of the most important advantages associated with a device according to the invention is that the patient can check blood glucose levels throughout the day and, through experience, a familiarity can be built up with the patterns of fluctuation in blood glucose level associated with normal daily routine and with extraordinary events such as strenuous exercise, the consumption of different types of foods and drinks and variations in insulin dosage. This will provide a diabetic with an awareness of the effect of various factors on his or her blood glucose levels and preventive action can be taken before it is strictly required. Developing such an association need not be a conscious exercise on the part of the diabetic, because an association of this type is built up through experience.

Heretofore, diabetic subjects have been able to recognise that blood glucose levels should be increased or decreased, but this is generally as a result of the onset of hyperglycemic or hypoglycemic symptoms. By recognising that corrective action is required before such symptoms develop, the blood glucose levels of the subject will be far more regular.

An additional advantage is that a diabetic subject using a device according to the invention will not mistake unrelated symptoms as being related to abnormally high or low blood glucose levels. An objective check is available which prevents the subject from mistakenly increasing insulin or sugar intake.

Although not explicitly enumerated, many of the features of the invention in its first aspect are suitable for incorporation into the second aspect, as will be apparent to the skilled person. Furthermore, both aspects of the invention can be combined to provide a delivery device with monitoring and display features.

In a third aspect, the invention provides a method of measuring the plasma concentration of an analyte comprising the steps of: a) penetrating the epidermis with an enzymatic sensor which forms part of an electrical circuit,

wherein the current through the circuit is dependent on the presence of a species produced by the enzymatic reaction with the analyte; b) supplying a periodic potential to the enzymatic sensor such that current only flows through the electric circuit intermittently; and c) measuring the current shortly after it begins to flow.

For the reason indicated above and, as will be further illustrated below, this method has been found to provide accurate results in a far simpler and more efficient manner than the chronocoulometric method known from the prior art.

Suitably, the potential is supplied intermittently as a periodic stepped potential, providing a disconnect period and a connect period, thereby giving rise to a peak current at the beginning of the connect period, falling away towards a steady state current level.

In a presently preferred embodiment, the disconnect period is at least one second long and the connect period is at least 20 microseconds long.

Preferably, the connect period is in the range 20–400 microseconds. More preferably it is in the range 40–80 microseconds.

Further, preferably, the disconnect period is in the range 1–15 seconds, more preferably 5–10 seconds.

These periods have been found to provide good results when used with the type of glucose sensor further described below. The disconnect period should be long enough for a substantial amount of the current-dependent species to build up at the electrode, in order to provide a strong peak current at the beginning of the connect period.

Suitably, the current is measured in the first 15 microseconds of the connect period. Preferably, the current is measured between 0.25 and 10 microseconds after the beginning of the connect period, and most preferably between 0.5 and 3 microseconds after the beginning of the connect period.

By measuring the current early in the connect period, a strong peak current will be obtained, thereby boosting the signal to noise ratio relative to a steady state amperometric measurements. In the method described by Rishpon (supra), measurements were only made every 200 microseconds. It has been found that the best results are obtained if measurements are made well within 200 microseconds of the start of the connect period, as after 200 microseconds the current will have effectively dropped to a steady state level for many constructions of electrode.

As indicated above in relation to the device, preferably, the method further comprises the steps of measuring the current a second time during the connect period, calculating a ratio between the two measured values, and comparing this ratio to a memorised value or range of values to determine whether the sensor is performing normally. Preferably the second current measurement is made when the current has fallen to a steady-state value.

Suitably, the method also comprises the step of providing an indication that the sensor is defective if the calculated ratio is different to the memorised value or range of values.

This indication can be effected in many ways, preferably by providing a visible or audible alarm.

BRIEF DESCRIPTION OF THE FIGURES

The invention will be further illustrated by the following description of embodiments thereof, given by way of example only with reference to the accompanying drawings, in which:

FIG. **1** is a cross-section through a liquid delivery device according to the invention;

FIG. **2** is a magnified view of a detail of the device of FIG. **1**;

FIG. **3** is a cross-section through a second liquid delivery device according to the invention;

FIG. **4** is a view of the underside of the device of FIG. **3**;

FIG. **5** is a schematic representation of the electronic circuit of the device of FIG. **3**;

FIG. **6** illustrates an alternative construction of sensor needle for use in a device according to the invention;

FIG. **7** illustrates a detail of the sensor needle of FIG. **6**;

FIG. **8** is a schematic cross section through an embodiment of a device for monitoring plasma glucose levels, according to the second aspect of the invention;

FIG. **9** is a plan view of the underside of the device illustrated in FIG. **8**;

FIG. **10** is a perspective view of an actual device of the type schematically illustrated in FIG. **8**, before assembly;

FIG. **11** is a perspective view of the device of FIG. **10** when assembled;

FIG. **12** is a diagram of the potential applied to the sensor electrode and the corresponding current obtained from the electrode;

FIG. **13** is a plot of actual current profiles achieved for different glucose concentrations;

FIG. **14** is a plot of instantaneous current values against glucose concentration showing a linear relationship between current and glucose concentration;

FIG. **15** is a plot of the ratio of two instantaneous current readings taken at different times for various glucose concentrations in respect of three different electrodes, showing how this ratio can be used to evaluate the performance of the electrode;

FIG. **16** is a side cross sectional elevation of a further embodiment of sensor needle for use with the device according to the invention; and

FIG. **17** is a front elevation of the needle of FIG. **16**.

DETAILED DESCRIPTION OF THE INVENTION

FIG. **1** shows a device according to the invention, illustrated generally at **10**, for use in the controlled delivery of insulin to a "Type 1" diabetic subject (i.e. suffering from insulin-dependent diabetes mellitus).

The device **10** comprises a housing **11** containing an insulin reservoir **12** for storing insulin in liquid form (suspension, solution or liquid) and a gas generation chamber **13**. Reservoir **12** and gas generation chamber **13** are separated by an elastomeric membrane **14**, such that an expansion of gas generation chamber **13** leads to a corresponding contraction of insulin reservoir **12**.

A platinum-iridium delivery needle **15** projects through a lower surface **16** of housing **11** by a distance of 2.5 mm. Delivery needle **15** is hollow and is open at an inner end **17** to insulin reservoir **12**. It is also open at outer end **18** such that, when lower surface **16** of housing **11** is pressed against a subject's skin, delivery needle **15** penetrates through the epidermis and the dermis, thereby establishing communication between insulin reservoir **12** and the subject's subcutaneous tissue via the hollow needle **15**. If a shorter needle is used, communication can be established with the capillary system of the dermis.

Gas generation chamber **13** is provided with an electrolytic cell **19** powered by a battery **20** under the control of a programmable microprocessor **21**. Microprocessor **21** controls the rate at which gas is generated in electrolytic cell **19** by the electrolysis of water.

Electrolytic cell **19** has walls of a hydrophobic material which allow gas to permeate therethrough but which retain water within the cell. When gas is generated by electrolytic cell **19**, the pressure increases in gas generation chamber **13**, causing the volume of chamber **13** to expand with a corresponding contraction of insulin reservoir **12**, resulting in insulin being forced out of reservoir **12** through needle **15** (and, in use, into the patient's tissue).

Microprocessor **21** controls the rate of gas generation and, consequently, the rate of insulin delivery, by monitoring the patient's blood glucose level by means of a glucose sensor, indicated generally at **22**. Sensor **22** comprises a platinum-iridium sensor needle **23** extending from lower surface **16** by about 2 mm.

Referring additionally to FIG. **2**, it can be seen that sensor needle **23** is hollow and is open at both ends. Inner end **24** leads to a passageway **25** extending through housing **11** to the external atmosphere. Accordingly, outer end **26** of sensor needle **23** is, via passageway **25**, in communication with a supply of excess oxygen. Needle **23** is coated with a glucose oxidase enzyme coating **27**. This entire composite needle structure is covered by a layer of "Nafion" **28** which serves as a protective material, but is permeable to glucose, water, oxygen and hydrogen peroxide. "Nafion" layer **28** also covers open end **26** of stainless steel needle **23**, thereby stopping blood from entering and filling the hollow interior of needle **23**.

Oxygen within sensor needle **23** can diffuse through "Nafion" coating **28** into glucose oxidase enzyme layer **27**. In addition, glucose and water can also diffuse through "Nafion" layer **28** into glucose oxidase enzyme containing layer **27**. The enzyme catalyses the reaction of glucose with oxygen and water, producing gluconic acid and hydrogen peroxide. Accordingly, hydrogen peroxide is produced in enzyme layer **27** surrounding platinum-iridium needle **23** in an amount which is directly dependent on the amount of available glucose in the bloodstream.

Delivery needle **15** is a coated with a silver/silver chloride layer. Battery **20** is connected between delivery needle **15** and sensor needle **23** via internal connecting wires **31** (FIG. **2**) within the housing. Accordingly, when the needles **15**,**23** penetrate into the dermis or the subcutaneous tissue, a circuit is closed by the establishment of an electrical connection between the needles **15**,**23**. The circuit is effectively an electrochemical cell, with one electrode being a standard silver/silver chloride electrode in aqueous solution (i.e. needle **23** with its coating immersed in the bloodstream) and the other electrode being a platinum electrode supplied with hydrogen peroxide.

The free mobile charges providing a flow of current through the sensor needle are produced in the catalysed oxidation of hydrogen peroxide on platinum in the reaction:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

The electrons produced in this reaction allow current to flow through the sensor needle **23**.

The current through the circuit is limited by the numbers of electrons available at sensor needle **23**. This means that, since the electrons are produced by hydrogen peroxide oxidation and the hydrogen peroxide is produced by the enzymatic oxidation of glucose, that the current depends on the glucose concentration in the bloodstream.

The current through the circuit is amplified and measured by microprocessor **21**. Microprocessor **21**, which comprises a stored programme, calculates the precise amount of insulin which must be delivered at any time in order to maintain glucose at the optimum physiological concentration.

The microprocessor **21** maintains this concentration by controlling the current flowing through electrolytic cell **19**, since any increase or decrease in the amount of gas produced by electrolytic cell **19** results in a corresponding increase or decrease in the amount of insulin injected into the subject via needle **15**. In effect, therefore, device **10** acts as an artificial pancreas which continually monitors the glucose concentration in the bloodstream and constantly adjusts the on-going rate of insulin administration to take account of the measured glucose level.

In contrast to prior art devices for the administration of insulin, device **10**, which can be affixed to any suitable area of the skin (such as the upper arm or abdomen) is unobtrusive. It is easy and painless to apply; simply by pressing lower surface **16** against the skin, the two needles **15**,**23** penetrate the skin and an adhesive layer **29**, which is provided on lower surface **16**, holds the device in place throughout the course of treatment. A device having a diameter of approximately 5 cm and a thickness of approximately 1 cm may contain a sufficient amount of insulin for treatment throughout 12 hours, 1 day, or up to 1 week.

The insulin used in the device may be chosen to meet the requirements of the patient. It may be bovine, porcine, human or synthetic and it may be short acting or long acting, or it may comprise a mixture of different types of insulin.

The needles **15**,**23**, including the coatings thereon, have an external diameter of 0.2 mm. Accordingly, there are no large, open wounds (as there are with traditional delivery cannulas and sensor implants) which may become infected. Additionally, since the site of application can be changed daily, for example, the wounds will heal almost immediately and there is no possibility of either the sensor needle or the delivery needle becoming coated with fibrin.

A preferred embodiment of the invention is illustrated in FIG. **3**. The device, indicated generally at **40**, comprises a housing **41** containing an insulin reservoir **42** and a gas generation chamber **43** within which there is provided an electrolytic cell **44**. Reservoir **42** and gas generation chamber **43** are separated by an elastomeric membrane **45** such that when gas is generated by electrolytic cell **44**, gas generation chamber **43** expands by displacing membrane **45** downwards and thereby contracting insulin reservoir **42** causing the drug to be discharged therefrom.

The rate of generation of gas is controlled by a microprocessor **46** which receives a signal from a glucose sensing apparatus comprising a potentiostat **47** linked to a sensor needle **48** and a delivery needle **49** of the types described above with reference to FIGS. **1** and **2**. The potentiostat is also connected to a reference electrode **50** on the lower (skin-contacting) surface **51** of housing **41**. The arrangement of sensor needle **48**, delivery needle **49** and reference electrode **50** on lower surface **51** of housing **41** as illustrated in FIG. **4**.

FIG. **5** is a schematic representation of the electronic circuit of device **40**. Potentiostat **47** is shown as a dotted outline. It comprises an operational amplifier **51**, a power source (Vin) connected between the positive input of operational amplifier **51** and sensor needle **48** (which acts as the working electrode), and a resistor **52** connected between the output of operational amplifier **51** and delivery needle **49** (which acts as the counter electrode). Reference electrode **50** is connected to the negative input of the operational amplifier **51**.

Potentiostat **47** serves to hold the working electrode **48** at a fixed potential relative to the reference electrode. Since both inputs to the operational amplifier are effectively at the same potential, the potential difference between reference

electrode **50** and working electrode **48** is equal to Vin. The current through the amplifier **51**, which is dependent on the amount of glucose detected by sensor needle **48**, is effectively independent of the resistance of the "cell" between working electrode **48** and counter electrode **49**, (at least within the operating range of the operational amplifier).

The current is calculated from the voltage drop across resistor **52** using a voltmeter **53** which provides a signal to microprocessor **46** which interprets the signal as indicating a certain glucose concentration in the tissue surrounding sensor needle **48**. Voltmeter **53** actually includes both a floating-input voltmeter and an amplifier connected to the output of the voltmeter to provide a signal of suitable strength to microprocessor **46**. One or more power sources (not shown) are also included for the purposes of powering electrolytic cell **44** (FIG. **3**), the amplifier connected to the voltmeter output, and microprocessor **46**. The power source (s) may be that/those used in the potentiostat circuit or separate power source(s) may be provided.

An alternative composition of sensor needle to that illustrated in FIG. **2** is shown in FIG. **6**. A hollow stainless steel sensor needle **60** has a single coating layer **61** formed from a casting solution of perflourosulphonic acid polymer, such as the perflourinated ion exchange membrane, "Nafion", glucose oxidase enzyme, and a carbon supported catalyst.

As illustrated in more detail in FIG. **7**, the "Nafion" membrane **62** provides an insoluble biocompatible protective matrix for the enzyme **63** retains the enzyme for long term availability in the electrode structure. Membrane **62** also dissolves large quantities of oxygen that is then available adjacent to the enzyme to promote hydrogen peroxide formation for signal generation. The carbon supported catalyst is in the form of platinum-loaded carbon particles **64** having about 10% by weight of platinum. The particles **64** serve two functions: firstly, the catalytic surface for oxidation of hydrogen peroxide is dispersed throughout the matrix layer **62** within which the hydrogen peroxide is generated; secondly, the carbon support for the catalyst provides an electrically conductive path for electrons produced by the oxidation reaction. An electrode having this type of supporting layer is described in U.S. Pat. No. 5,227,042, the disclosure of which is incorporated herein by reference. As U.S. Pat. No. 5,227,042 discloses, other catalysts from the platinum group, such as palladium, ruthenium or rodeium can be used in place of platinum.

FIG. **8** is a schematic illustration of an embodiment of the second aspect of the invention, namely a device for monitoring the plasma concentration of an analyte. The device, indicated generally at **50**, comprises a housing **51** detachable into a first part **52** and a second part **53**. The device **50** has a number of features in common with the embodiments of the first aspect of the invention. Specifically, first part **52** of housing **51** has an adhesive lower surface **54** which is provided with a working electrode **55**, a counter electrode **56** and a reference electrode **57**. The electrodes **55**,**56**,**57** are connected to a potentiostat **58** as hereinbefore described.

Working electrode **55** is a platinum-iridium needle coated with a glucose oxidase enzyme coating, as previously described. Counter electrode **56** is in the form of a platinum-iridium surface adapted to rest against the subject's skin and reference electrode **57** is in the form of a silver/silver chloride surface adapted to rest against the subject's skin. As previously described, the current passing between working electrode **55** and counter electrode **56** provides a measure of the glucose concentration in the vicinity of working electrode **55**. This current is measured by a microprocessor **59** which is calibrated to allow calculation of the glucose plasma concentration from the measured current through potentiostat **58**.

Microprocessor **59** is pre-programmed to activate an audible alarm **60** in the case of hyperglycemia or hypoglycemia. These conditions are recognised by the microprocessor if the calculated glucose concentration rises above or falls below a specific range. Alarm **60** emits different sounds depending on whether hyperglycemia or hypoglycemia is indicated by microprocessor **59**. In practice, microprocessor **59** activates audible alarm **60** before the glucose plasma concentration reaches a dangerous level. Thus, the subject, or those supervising the subject, can act in good time by administering glucose-rich food and drink or by administering insulin, as the case may be, before corrective action becomes absolutely critical.

Microprocessor **59** also communicates with a liquid crystal display (LCD) **61** which has seven-segment displays to provide a numerical indication of the level of glucose in the subject's plasma. Thus, if device **50** is worn on a continual basis, the subject can check his or her blood glucose levels at will. In this way, the subject can titrate insulin and/or sugar intake as and when required to provide a plasma glucose profile which more closely resembles that of a healthy individual than that of a self-administering diabetic who self-administers insulin according to traditional criteria (i.e. fixed dosages, variable dosages according to the results of occasional blood tests).

Whereas blood tests prior to insulin administration can allow patients to determine optimum dosages, it is impossible for a diabetic to objectively gauge his or her glucose intake requirements between injections, so the diabetic subject is either confined to a strictly controlled diet or else runs the risk of misjudging a safe level of sugar intake.

Battery **62** powers the device and a start button **63** is provided to activate the device after administration to the skin of the subject.

As illustrated, the device **50** is in two parts **52**,**53** which are separable from one another. First part **52**, which is disposable, comprises the three electrodes **55**,**56**,**57** and lower surface **54**. As the efficiency of the electrodes will decrease over time (in particular, the dependability of the enzymatic sensor or working electrode **55** will not remain stable indefinitely), it is desirable to replace the electrodes on a regular basis. Second part **53** houses all of the reusable elements of the device. Electrical contact is effected between potentiostat **58** and electrodes **55**,**56**,**57** by means of two sets of interengagable contacts **64**,**65** which fit together when first part **52** is mounted on second part **53**. Thus, first part **52** can be replaced daily, for example, whereas second part **53** can be reused indefinitely.

Suitably, battery **62** is a long-term battery which allows second part **53** to operated continuously over two-three years before replacement of battery **62** is necessitated. Microprocessor **59** monitors the power level of battery **62**. As battery **62** becomes exhausted, its power decreases and microprocessor **59** activates alarm **60** to provide a special alarm indicating that replacement of battery **62** is necessary.

Push button **63** performs an additional function in that it can be used to reset the alarm when blood glucose levels have moved outside the acceptable range; microprocessor **59** will then only reactivate alarm **60** when calculated glucose levels next move outside the allowable range or, if the levels do not return to normal, when the patient's plasma glucose levels worsen appreciably.

FIG. **9** shows a view of the underside of device **50**. Thus, lower surface **54** of first part **52** is seen with working electrode **55** (i.e. the enzymatic sensor needle) in the centre.

On either side, two approximately semi-circular surfaces **56**,**57** are indicated by shaded lines. Surface **56** is the platinum-iridium surface of the counter electrode, while surface **57** is the silver/silver chloride surface of the reference electrode. Lower surface **54** is provided with a suitable adhesive to hold device **50** securely in place against the subject's skin.

In FIGS. **10** and **11**, device **50** of FIGS. **8** and **9** can be seen in perspective view. FIG. **10** shows first and second parts **52**,**53** before assembly. First part **52** has three contacts **65** on the upper surface thereof which receive three complementary contacts (not shown) on the lower surface of second part **53**. As indicated in FIG. **11**, second part **53** is provided with a liquid crystal display **61** which gives a numerical indication of the blood glucose levels. Beside LCD **61**, push button **63** can be seen. An additional feature which is not illustrated in FIGS. **8** and **9** is a release liner **64** which covers the lower surface (not visible) of first part **52** before use. Release liner **64** is provided both for safety reasons (i.e. to cover the needle before use) and to ensure that the electrode surfaces are undamaged upon application to the skin of the subject.

FIG. **11** shows device **50** when first part **52** has been mounted on second part **53** to form a single housing **51**. First and second parts **52**,**53** are held together by means of a snap action mechanism (not shown). In use, release liner **64** is then removed and housing **50** is present against the surface of the subject's skin such that the sensor needle (not shown) penetrates through the epidermis and into the dermis (depending on the length of the sensor needle, it may also penetrate through the dermis to the subcutaneous tissue) to allow contact between the enzymatic coating on the sensor needle and the subject's plasma. Contact is also effected between the subject's skin and each of the flat electrodes. Operation of the monitoring device begins when push button **63** is pressed. At the end of 24 hours, first part **52** is snapped away from second part **53** and replaced by a new, identical part for monitoring blood glucose levels throughout the subsequent 24 hour/period.

The operation of the measurement circuit has been described above with the working electrode held at a constant potential above the reference electrode. In a more sophisticated embodiment of the invention, however, a potential is only applied intermittently to the working electrode, as indicated in FIG. **12**. From FIG. **12** it can be seen that the potential is stepped between a lower value (preferably 0.0 V) where no current will flow, and a higher value (such as 0.6 V) where current is allowed to flow. FIG. **12** is not to scale and the disconnect period is preferably many times longer than the connect period. In a preferred embodiment, the disconnect period is 3–12 seconds and the connect period is 20–300 microseconds. In the experiments described below, the disconnect period was 7 seconds and the connect period was 60 microseconds.

During the disconnect period, the enzymatic reaction proceeds and hydrogen peroxide builds up at the sensor electrode. Because no potential has been applied and no current is flowing, however, the hydrogen peroxide accumulates continually until the potential is applied allowing a current to flow. As can be seen in the upper half of FIG. **12**, the current begins with a peak which then falls away as the hydrogen peroxide is consumed.

Referring additionally to FIG. **13**, actual curves obtained using this method can be seen. From these curves it will be seen that the peak value is many times greater than the steady state value achieved after, for example, 30 microseconds. If one compares the peak values obtained for glucose concentrations of 0, 1, 2, 4 and 8 mM it can be seen that one can easily distinguish between and measure the peak concentrations, whereas the steady state concentrations are so close together as to be almost indistinguishable. Thus, a greatly improved signal to noise ratio is obtained by applying an intermittent voltage and measuring the current obtained at the peak (or shortly thereafter). This greatly enhances the accuracy of measurements which can be made using this type of enzymatic sensor, and it has been found that the response of peak current to glucose concentration is effectively linear. The measurements in FIG. **14** were taken one microsecond after the potential was applied. Each data point therefore represents a single current reading at t=1 ms μs for a given glucose concentration.

The performances of the electrodes were evaluated by measuring the currents I_1 at t=1 μs and I_2 at t=55 μs and then calculating the ratio I_1/I_2. This ratio was calculated for each glucose concentration for three different electrodes. One of the electrodes (experiment **101**) had degraded and had lost its ability to measure glucose properly, whereas the other two electrodes (experiments **111** and **112**) were functioning perfectly. It can be seen that the ratio I_1/I_2 in each case is independent of glucose concentration and is equal for the electrodes used in experiments **111** and **112**. However, a lower value for I_1/I_2 was obtained in experiment **101** and is indicative of the loss of performance. While more sophisticated analytical techniques can be based on the principle used to make the FIG. **15** measurements, FIG. **15** represents a very simple but effective method of continuously monitoring electrode performance. The detecting circuit can be designed to sound an alarm or provide a visual indication when the ratio I_1/I_2 changes by an appreciable amount, thereby indicating that the electrode should be replaced.

For best results it has been found advantageous to measure I_1 as quickly as possible (e.g. 1–1.5 μs after the circuit is closed) and to measure I_2 when the current has reached steady state (e.g. after 90% of the total connect time has elapsed).

In summary, the "pulse sampling method" described above and illustrated by FIGS. **12**–**15** provides the following advantages:

(i) the signal is at least two orders of magnitude higher than with a continuous sampling method. Therefore, less amplification is needed, and higher accuracy is achieved.

(ii) the signal to noise ratio is vastly improved; the noise obtained is less than 10% of the signal. In the continuous sampling (or continuous current) method, the noise is higher than the signal itself and its value is eliminated by averaging the samples.

(iii) the pulse sampling method is less sensitive to the presence of substances such as ascorbic acid, uric acid, paracetamoi, etc. The reason for this is that enzymatic detection is effected using two reactions: firstly, the chemical reaction where the analyte is converted and a by-product such as hydrogen peroxide is formed; and secondly, an electrochemical reaction, where hydrogen peroxide is consumed and an electric current flows through the electrodes. The chemical reaction takes place whenever the reactants and enzyme are present, while the electrochemical reaction only takes place when the electrode is at a sufficient potential. At that potential, the abovementioned substances also react with the electrode and induce an undesired current that adds to the current generated by hydrogen peroxide decomposition. When using the pulse sampling method, the voltage is intermittently connected to the

electrodes. When disconnected, hydrogen peroxide accumulates at the electrode site but the other substances do not accumulate appreciably during these breaks. Therefore, when reconnecting the potential, there is an "amplification" of the hydrogen peroxide signal compared to the signal resulting from the other substances and therefore their contribution to noise becomes less significant. This "amplification" of the hydrogen peroxide signal relative to the ascorbic acid/uric acid/paracetamol signals would not exist if the voltage was applied continuously.

(iv) The values, gradient and shape of the pulse carry important information about the condition of the sensor. This can be used to monitor the sensor. In prior art electrodes, the degradation of the sensor would only show as an artificially increased or decreased analyte measurement which would be more likely to be wrongly interpreted as an actual measurement than to be interpreted as an indication of sensor degradation. The ability to distinguish between a false signal and a damaged sensor means that the sensor according to the invention is far safer than known sensors for use as a measuring tool.

In FIGS. **16** and **17**, there is indicated, generally at **70**, a further embodiment of sensor needle for use with a device according to the invention. The needle **70** comprises a platinum-iridium rod **71** having a bevelled tip **72**. The rod **71** is 0.3 mm in diameter.

A transverse bore **73** extends through the thickness of the rod, and bore **73** is filled with an enzyme matrix **74** formed from a casting solution of perflourinated ion exchange membrane ("Nafion"), and glucose oxidase enzyme. A bore **75** extends axially through the length of the rod allowing communication between enzyme matrix **74** and the atmosphere. Bore **75** is 0.1 mm in diameter. Each of bores **73** and **75** can be conveniently formed by laser drilling.

Needle **70** works in exactly the same manner as the needles previously described, but provides an advantage in that the enzyme matrix **74** is provided internally of the needle and not as an external coating. This eliminates any tendency for the enzyme layer to be damaged or scratched during manufacture (i.e. when the needle is affixed to the body of the device).

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed because these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A device for monitoring the concentration of an analyte in a subject, comprising:

a housing having a lower surface for application to the skin of the subject;

means for holding the housing in position with the lower surface against the subject's skin;

an electrical detection circuit comprising a power source connected across two electrodes mounted on said lower surface and switching means, the circuit being completed upon application of the lower surface to the skin of the subject, one of said electrodes being a sensor needle for penetrating through the epidermis and into the dermis when the lower surface is applied to the skin and having an enzyme associated therewith, said enzyme being specific to the analyte to be detected, and the switching means allowing the current through the circuit to flow intermittently to the sensor needle; and

a communication circuit comprising means for measuring the current through said electrical detection circuit, means for calculating the concentration of the analyte from the measured current and communicating means for communicating the calculated concentration to the subject.

2. A device according to claim **1**, wherein the enzyme is glucose oxidase and the analyte to be measured is glucose.

3. A device according to claim **1**, wherein the sensor needle is a working electrode and the other of said two electrodes is a counter electrode in the form of a platinum surface for contact with the subject's skin.

4. A device according to claim **1**, wherein the electrical detection circuit also comprises a reference electrode on the lower surface of the housing, in the form of a silver/silver chloride surface for contact with the subject's skin, and a potentiostat having an operational amplifier which drives a current between the working electrode and the counter electrode.

5. A device according to claim **4**, wherein the power source and the sensor needle are connected in series with the positive input of the amplifier and wherein a resistor and the delivery needle are connected in series with the amplifier output, the reference electrode being connected to the negative input of the amplifier.

6. A device according to claim **5**, wherein the current through the circuit is determined by measuring the voltage drop across said resistor.

7. A device according to claim **6**, wherein a voltmeter connected across said resistor provides a signal determined by the magnitude of the voltage drop and the signal is amplified and supplied to the communicating means.

8. A device according to claim **1**, wherein the switching means comprises means for intermittently applying a voltage to the sensor needle.

9. A device according to claim **8**, further comprising means for comparing the current at different times.

10. A device according to claim **9**, wherein the means for comparing the current at different times is integral with the means for controlling the rate of active discharge.

11. A device according to claim **1**, wherein the sensor needle is provided with a conduit permitting communication between the inner skin-contacting end and a source of oxygen.

12. A device according to claim **11**, wherein the source of oxygen is the atmosphere.

13. A device according to claim **1**, wherein the housing comprises a first part and a second part, the first part comprising the lower surface and the electrodes and the second part comprising the power source and the communication circuit.

14. A device according to claim **13**, wherein the first part is detachably mounted on the second part, such that the first part can be disposed of and replaced and the second part can be reused a number of times.

15. A device according to claim **1**, wherein the communicating means is activated when the calculated analyte concentration falls outside a predetermined range.

16. A device according to claim **1**, wherein the communicating means comprises an audible alarm.

17. A device according to claim 1, wherein the communicating means operates continuously to provide a constant indication of the subject's analyte concentration.

18. A device according to claim 1, wherein the communicating means comprises a visible display of the analyte concentration.

19. A device according to claim 18, wherein the visible display is in the form of a liquid crystal display for indicating the analyte concentration as a numerical value.

20. The device of claim 1 wherein the concentration is communicated to the subject.

21. The device of claim 1 wherein the concentration is communicated to a microprocessor.

22. The device of claim 1 wherein the microprocessor is in communication with a liquid drug delivery device for delivering drug to the subject via the subject's skin, the device containing a liquid drug, whereby the microprocessor controls the rate of discharge of drug via the subject's skin in response to the communication thereto.

* * * * *

US005505694A

United States Patent [19]

Hubbard et al.

[11] **Patent Number: 5,505,694**

[45] **Date of Patent: Apr. 9, 1996**

[54] **APPARATUS AND METHOD FOR RAISING A SKIN WHEAL**

[75] Inventors: **Vance M. Hubbard**; **Welton K. Brunson**, both of Bedford; **V. C. Saied**, Wichita Falls, all of Tex.

[73] Assignee: **TCNL Technologies, Inc.**, Wilmington, Del.

[21] Appl. No.: **169,489**

[22] Filed: **Dec. 17, 1993**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 940,526, Sep. 4, 1993, abandoned, which is a continuation-in-part of Ser. No. 572,508, Aug. 22, 1990, Pat. No. 5,190,521.

[51] **Int. Cl.**[6] **A61M 31/00**; A61M 5/00

[52] **U.S. Cl.** **604/51**; 604/242

[58] **Field of Search** 604/112, 117, 604/192, 199, 201, 204, 206, 212, 240–242, 51, 905; 206/363–366, 368

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Primary Examiner—John D. Yasko
Assistant Examiner—Adam J. Cermak
Attorney, Agent, or Firm—Baker & Botts

[57] **ABSTRACT**

The sight of a large I.V. needle makes one shiver with fright due to one's association of an I.V. needle with pain. An apparatus and method of anesthetizing the intended site for I.V. needle insertion is provided to reduce or eliminate the pain. In addition, apparatus and method may be used for diagnostic testing purposes to reduce the amount of pain associated with this procedure. The apparatus (**10**) comprises a storage chamber (**12**) containing an anesthetizing agent (**22**) and coupled to a seat member (**14**). The seat member (**14**) further holds a needle (**16**) in place and pierces the storage chamber (**12**). Alternate embodiments of the apparatus (**10**) include a seat member (**46**, **56**) which allows the needle (**52**, **68**) to penetrate the storage chamber (**40**, **62**) just prior to use. The apparatus pierces the skin, injects the anesthetizing agent and induces a skin wheal at the intended I.V. site.

2 Claims, 6 Drawing Sheets

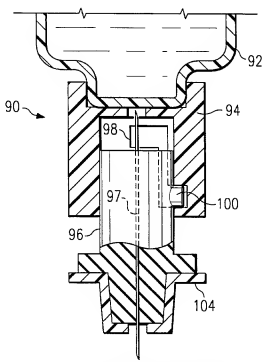


FIG. 1

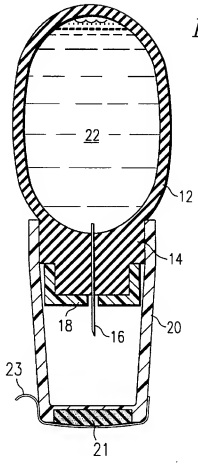
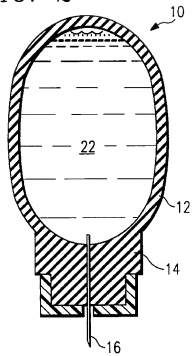


FIG. 2



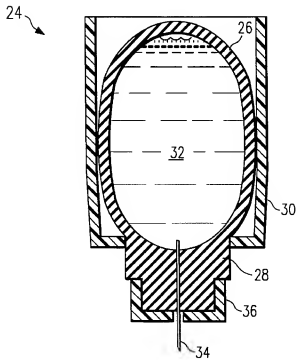


FIG. 3

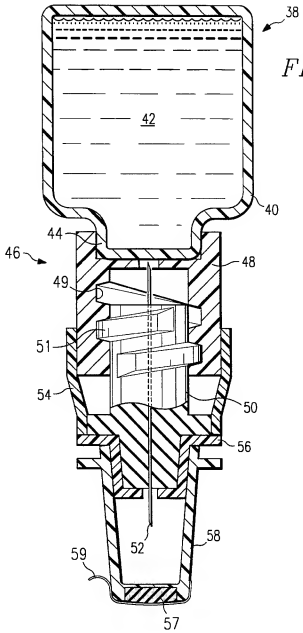

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FIG. 4

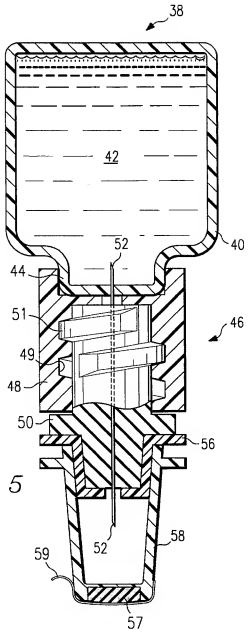
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FIG. 5

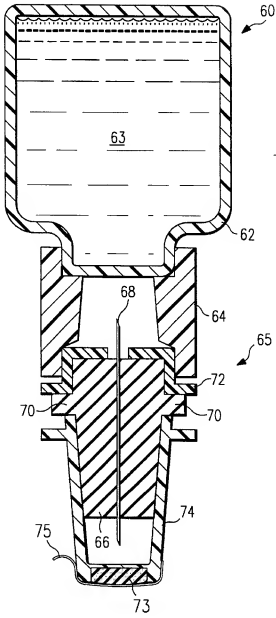

FIG. 6

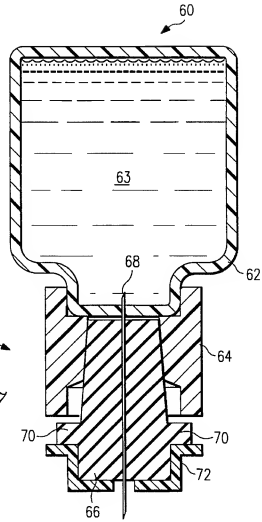

FIG. 7

FIG. 8

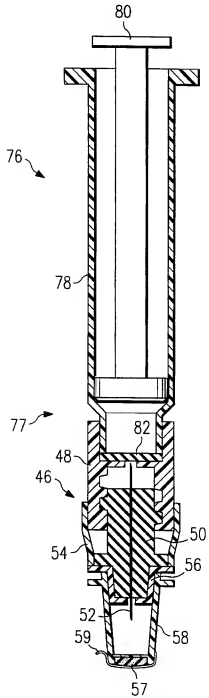

FIG. 9

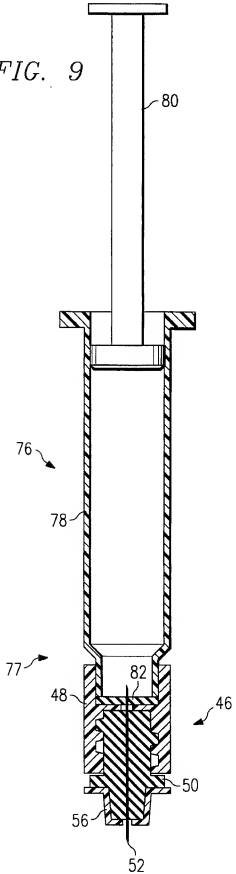

FIG. 10

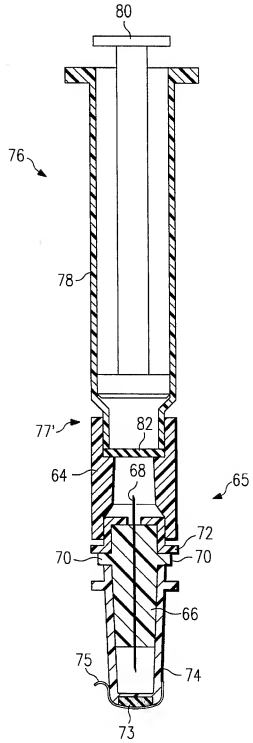
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FIG. 11

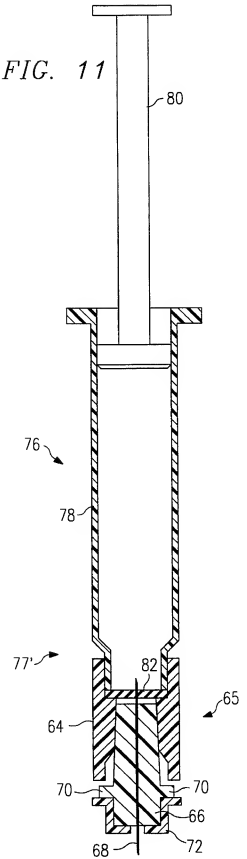
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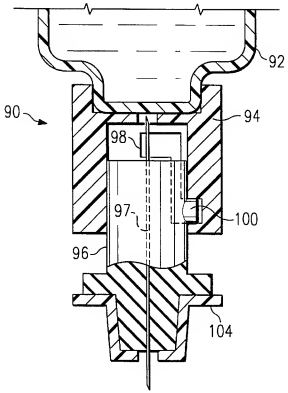
FIG. 12



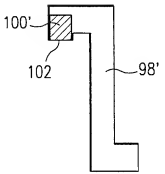


FIG. 13

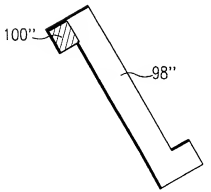


FIG. 14

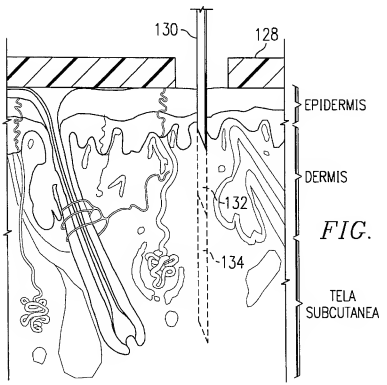


FIG. 15

APPARATUS AND METHOD FOR RAISING A SKIN WHEAL

RELATED APPLICATIONS

This application is a continuation-in-part of application Ser. No. 07/940,526, (Attorney Docket No. 26320-236) filed Sep. 4, 1993, by Vance M. Hubbard, Welton K. Brunson and V. C. Saied entitled "Apparatus and Method of Anesthetizing Skin," now abandoned, which is a continuation-in-part of application Ser. No. 07/572,508, (Attorney Docket No. 26320-1150), filed Aug. 22, 1990, by Vance M. Hubbard, Welton K. Brunson and V. C. Saied entitled "Apparatus and Method of Anesthetizing Skin," now U.S. Pat. No. 5,190,521, issued Mar. 2, 1993.

TECHNICAL FIELD OF THE INVENTION

This invention relates in general to medical products, and more particularly to an apparatus and a method for anesthetizing skin.

BACKGROUND OF THE INVENTION

The insertion of an intravenous (I.V.) catheter can be a frightening and painful experience. The sight alone of the large gauge needle typically used in an I.V. catheter is enough to cause the patient to become anxious and tense. The patient's reaction causes his muscles to become tense and hard, making needle penetration difficult and painful.

Typically, the I.V. needle must be inserted into a vein along the forearm or on the back of a hand. It is not uncommon for a first attempt to insert the I.V. needle to fail because it is difficult to locate a vein in some patients. As a result, further painful probing is required to locate the vein and to insert the I.V. needle.

After insertion, an I.V. catheter may remain in place for an extended period of time. The site of penetration may remain painful due to the pulling and stretching of the catheter. As a result, the patient is subject to pain and suffering repeatedly and needlessly.

Accordingly a need has arisen to provide an apparatus to numb the intended I.V. needle penetration site in order to lessen the pain and suffering usually associated with I.V. catheter insertions. The present invention anesthetizes the general area around the needle penetration site and induces a skin wheal for painless of I.V. insertion. Where conventional syringes inject anesthesia into the tissues underlying the skin, the present invention is adapted for depositing the anesthesia in the skin to raise the skin wheal. The present invention is further directed to overcoming one or more of the problems as set forth above.

SUMMARY OF THE INVENTION

In accordance with the present invention, an apparatus and method for anesthetizing skin is provided which substantially eliminates or reduces pain introduced by I.V. catheter administration.

In one aspect of the present invention, an apparatus for anesthetizing skin is provided. The apparatus comprises a storage chamber for containing an anesthetizing agent, means for injecting the anesthetizing agent into the skin and a spacing element for determining the depth of injecting means penetration into the skin.

In another aspect of the present invention, an apparatus for anesthetizing skin prior to an I.V. needle insertion is provided. The apparatus comprises a storage chamber for containing an anesthetizing agent and a seat member coupled to the storage chamber at one end. It further comprises a needle arranged for piercing the storage chamber wall and acquiring the anesthetizing agent. To vary the amount of needle penetration, a detachable spacer is coupled to the seat member. There is also provided a cap detachably coupled to the apparatus for shielding the needle from contaminants.

In yet another aspect of the present invention, there is provided a method for anesthetizing skin comprising the steps of pricking the section of skin with an injector, perpendicularly penetrating the injector into the skin, and injecting an anesthetizing agent therein to form a skin wheal.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing, and additional objects and advantages of the invention will become more apparent as the following detailed description is read in conjunction with the accompanying drawing wherein like reference characters denote like parts in all views and wherein:

FIG. **1** is a fragmentary view of apparatus constructed in accordance with the present invention;

FIG. **2** is a view similar to FIG. **1**, but showing the apparatus of FIG. **1** when it is ready for use;

FIG. **3** is a cross-sectional view of a modification of the apparatus of FIG. **1** that is also constructed in accordance with the invention;

FIG. **4** is a cross-sectional view of another embodiment of the apparatus that is constructed in accordance with the invention;

FIG. **5** is a view similar to FIG. **4**, but showing the apparatus as it is ready for use;

FIG. **6** is a cross-sectional view of still another embodiment of the invention that is also constructed in accordance with the invention;

FIG. **7** is a view similar to FIG. **6** showing the apparatus of FIG. **6** as it is ready for use;

FIG. **8** is a cross-sectional view of a modification of the apparatus of FIG. **4** for use with a intradermal syringe that is also constructed in accordance with the invention;

FIG. **9** is a view similar to the view of FIG. **8** showing the apparatus of FIG. **8** as it is ready for use;

FIG. **10** is a cross-sectional view of a modification of the apparatus of FIG. **6** that is also constructed in accordance with the invention;

FIG. **11** is a view similar to FIG. **10** showing the apparatus of FIG. **10** as it is ready for use;

FIG. **12** is a partial fragmentary view of yet another embodiment of the present invention employing an alternate twist-lock mechanism;

FIG. **13** is a unfurled view of another construction of the groove structure of the twist-lock mechanism;

FIG. **14** is yet another unfurled view of another construction of the groove structure; and

FIG. **15** is a cross-sectional side view showing the angle of needle penetration and the skin layers penetrated.

DETAILED DESCRIPTION OF THE INVENTION

With reference to the drawings, FIG. **1** and FIG. **2** show an embodiment of an apparatus for anesthetizing skin con-

structed in accordance with the teaching of the present invention. The apparatus, indicated generally at **10**, is shown in storage prior to use in FIG. **1** and ready for use in FIG. **2**.

The apparatus **10** comprises a storage chamber **12**. The storage chamber **12** may be made of a pliable and flexible material such as rubber, plastic and the like so that the wall of the storage chamber **12** may be compressed to decrease the volume thereof. One end of the storage chamber **12** is fixedly attached to a seat member **14** made of a rigid material such as rubber, plastic and the like. The rigid seat member **14** further accommodates a needle **16** located along its longitudinal axis and maintains a fixed spatial relationship between the storage chamber **12** and the needle **16**. The needle **16** is hollow to allow the passage of fluids and extends beyond the seat member **14** for inflicting a slight skin penetration. In the present embodiment of the instant invention, the needle **16** is shown penetrating the storage chamber **12** through the chamber wall even prior to use. It is preferable that the needle **16** is very fine, such as 23 gauge or less, so that the least amount of pain is inflicted on the patient. Furthermore, the fine-gauge needle **16** does not conduct the fluid contained in the storage chamber **12** unless the fluid is forced out by applying pressure to the chamber wall.

The human body experiences anatomical changes as it matures. One of the changes in the skin is the thinning of the various tissue layers. Skin thickness also varies on each person depending on its location on the body. In order to accommodate the difference in skin thickness, a spacer **18** is provided to vary the depth of needle penetration into the skin. The spacer **18** is coupled to the seat member **14** to decrease the length of the needle **16** available for skin penetration. To increase the depth of penetration, the spacer **18** may be removed to expose more length of the needle **16**. The thickness of the spacer **18** is determined by the range of skin thickness variations found in the general population, where skin thickness is generally determined by age, weight, anatomical site and other factors. The spacer **18** may be made of similar material as the seat member **14** and may be either rigid or slightly flexible.

The apparatus **10** further includes a cap **20** detachably secured over the needle **16**. The cap **20** shields the needle **16** from contaminants in the environment and keeps it sterile until the apparatus **10** is ready to be used. After use, the cap **20** safely contains the used needle point to protect the medical personnel and others from coming into contact with it. The cap **20** may be made of materials such as plastic.

Additionally, an antiseptic applicator **21** may be coupled to the tip of the cap **20**. The preferred embodiment of the applicator **21** is in the form of a sponge substantially saturated with an antiseptic solution, and secured within a cavity at the tip of the cap **20**. A foil **23** substantially seals the applicator **21** to prevent contamination and evaporation of the antiseptic solution. The foil **23** includes a portion which is graspable in order to facilitate tearing and removing the foil **23** from the antiseptic applicator **21** to expose the sponge. The antiseptic solution is applied to a site intended for I.V. penetration by rubbing the sponge to the skin.

The storage chamber **12** contains an anesthetizing agent **22** which may be of various compositions. Examples of the anesthetizing agent **22** are saline solutions, medicated saline solutions and local anesthetic solutions. It may be appreciated that in an alternate application, the storage chamber **12** may be filled with other types of solutions such as allergy testing compounds which are then introduced by the needle **16** into a patient in an allergy test.

FIG. **2** illustrates the apparatus **10** ready to be used. The cap **20** has been removed to expose the needle **16** and the seat member **14**. The spacer **18** is shown attached to the seat member **14** in this instance, but may be removed if circumstances so dictate.

Referring to FIG. **3**, a modification of the apparatus **10** is shown. The apparatus, indicated generally at **24**, is constructed substantially similarly to apparatus **10** except for differences described in detail below. A storage chamber **26** is coupled to a seat member **28** at one end. A housing **30** surrounds the storage chamber **26** and is also attached to the set member **28** at the same end. The housing **30** has an opening at the top to allow the entry of a finger or thumb to exert pressure on the storage chamber **26** during use. The housing **30** forms a substantially rigid shield around the storage chamber **26** to discourage discharge of the anesthetizing agent **32** through the needle **34** prior to usage. The seat member **28** and the housing **30** may or may not be of integral construction and the material thereof may or may not be alike. A spacer **36** may be further provided for determining the depth of penetration for the same purposes as enumerated above. A cap **20** and an antiseptic applicator **21** such as shown in FIG. **1** may also be included.

FIGS. **4** and **5** show another embodiment of the present invention. The apparatus, indicated generally at **38**, includes a storage chamber **40** for containing an anesthetizing agent **42**. The storage chamber **40** is shown constructed in a generally rectangular shape with a protruding portion **44**. It can be appreciated that the shape of the storage chamber **40** is not of great importance as long as the shape thereof facilitates the use of the apparatus **38** in accordance with the teaching of the present invention. The protruding portion **44** secures the storage chamber **40** to a seat member **46**.

Seat member **46** comprises a first and second portion **48,50**. The first portion **48** is substantially tubular with a cavity extending from one end of the first portion to the other end. A needle **52** is firmly implanted within the second portion **50**, with both ends extending beyond the ends of the second portion **50**, and with one end extending into the cavity of the first portion **48**. Thus the needle **52**, arranged in this fashion, is poised for penetration into the storage chamber **40**. The needle **52** may be made to pierce the storage chamber **40** by manipulating the first and second portions **48,50** of the seat member **46**. Preferably, the skin-piercing end of needle **52** is of a fine gauge in order to inflict as little trauma as possible. However, for ease of manufacturing, the entire needle **52** may be of the same fine-gauge size.

The first and second portions **48,50** are coupled together by a twist-lock or threaded mechanism as known in the art of coupling mechanisms. The first portion **48** has a spiraling groove **49** on its inner surface which mates with a spiraling ridge **51** on the outer surface of the second portion **50**. The second portion **50**, at a first position, is partially contained within the cavity of the first portion **48**. By rotatably twisting the second portion **50** with respect to the first portion **48**, the spiraling ridge **51** advances along the spiraling groove **49**, and the second portion **50** is advanced further into the first portion cavity to a second position, as shown in FIG. **5**.

Operated in this manner, the twist-lock arrangement allows the needle **52** to move from a first position apart from the storage chamber **40** to a second position, where it penetrates the storage chamber **40** walls and reaches the anesthetizing agent **42** contained therein. The ends of spiraling groove **49** may be tapered like that of the spiraling ridge **51** so that portion **50** may be "locked" in the first or

second positions by the added friction between ridge **51** and the walls of groove **49**.

Additional alternate embodiments of the twist-lock mechanism are discussed below in conjunction with FIGS. **12–14**. It can be appreciated that the twist-lock mechanism of the instant embodiment may be substituted by alternate coupling mechanisms and/or devices which perform substantially the same function to achieve substantially the same result. For example, the first and second portions **48,50** may be coupled by mating screw threads where the second portion **50** may be rotatably advanced to the second position; the second portion may be held in the first position by stop tabs, and the second portion may be advanced to the second position by forcibly pressing it into the first portion and breaking the stop tabs.

The second portion **50** and part of the first portion **48** of the seat member **46** are preferably encased in a casing **54**, which securely holds the second portion **50** in the first position and prevents the needle **52** from penetrating the storage chamber **40** prematurely. The casing **54** is removed prior to use. A spacer **56** is attachable to the second portion **50** and determines the penetration depth of the needle desired for the same reasons enumerated above. A detailed discussion of the spacer **56** may be found in association with FIG. **1**.

Further provided is a cap **58** which is attachable to the spacer element **56**. The cap **58** shields the needle **52** from the environment and keeps it sterile. An antiseptic applicator **57** is also provided, which includes a sponge saturated with an antiseptic solution and a protective foil **59** such as described above.

As shown in FIG. **5**, the apparatus **38** is ready for use. The second portion **50** has been advanced from the first position to the second position by twisting the second portion **50** with respect to the first portion **48**. As the second portion **50** advances to the second position, the needle **52** approaches the storage chamber **40** and eventually pierces it. Once the twist-lock mechanism locks the second portion in the second position, as shown in FIG. **5**, the cap **58** may be removed, and the apparatus is ready to be used for anesthetizing the general area intended for I.V. needle insertion. The apparatus **38** is shown with the spacer **56** in place.

Referring now to FIG. **6**, a third embodiment of the present invention is shown. The apparatus, indicated generally at **60**, comprises a storage chamber **62** made of a flexible and pliable material and contains a solution **63**. The storage chamber **62** is coupled to a first portion **64** of the seat member **65** which is generally tubular in shape. The first portion **64** of the seat member **65** has a first recess at one end for coupling to the storage chamber **62** and a second recess at the other end for coupling to a second portion **66**.

The second portion **66** is detachable from the first portion **64** of the seat member **65** and holds a needle **68** substantially along the longitudinal axis of the apparatus **60** and extends at both ends beyond the boundaries of the second portion **66**. The second portion **66** includes a stop **70** protruding generally perpendicularly from the longitudinal line of the apparatus **60** and serves as a stop to control the length of the second portion **66** which may enter the cavity of the first portion **64**. The stop **70** essentially divides the second portion **66** into two unequal sections, where the distance from one end to the stop **70** is longer than the distance from the other end.

The apparatus **60** further includes a spacer **72** detachably coupled to the shorter end of the second portion **66**. As shown in FIG. **6**, when the apparatus **60** is in storage, the shorter section of the second portion **66** is coupled to the first portion **64** along with the spacer **72** located therebetween. Arranged in this manner, the needle **68** is substantially spaced from the storage chamber **62**. A cap **74** is coupled to the second portion **66** and over the needle **68** to keep it sterile. An antiseptic applicator **73** is further provided and includes a sponge saturated with an antiseptic solution and a protective foil **75** sealing it from the environment.

The apparatus **60** in FIG. **7** has been readied for use. The cap **74** has been removed and the second portion **66** has been detached from the first portion **64** and reattached thereto by the other end. Since the stop **70** is located farther from the other end, the second portion **66** is able to enter the cavity of the first portion **64** more deeply, thereby allowing the needle **68** to pierce through the wall of the storage chamber **62**. The spacer **72** may remain attached to the second portion **66** to decrease the length of the needle **68** exposed and available for skin penetration.

Referring to FIG. **8**, there is illustrated apparatus **77** that is a modification of the apparatus **38** shown in FIG. **4**. The apparatus **77** includes a syringe-like element **76** having a hollow body **78** for holding a liquid. The hollow body **78** is further adapted to receive a plunger **80**, which is insertable therein at one end and may slidably advance and retreat along the longitudinal direction of the hollow body **78** to decrease and increase the effective volume of the hollow body **78**.

At the other end of the hollow body **78** is a stopper element **82** for containing the liquid within the hollow body **78**. The stopper element **82** may be made of materials such as rubber or plastic. Further coupled to syringe element **76** is a seat member **46** like that of the apparatus **38** shown in FIGS. **4** and **5**. Hereinafter, like numerals refer to like elements in FIGS. **4** and **5** to more clearly describe the structure and function of apparatus **77**. Seat member **46** has a first portion **48** coupled to a second portion **50**. The first and second portions **48,50** are coupled via a twist-lock mechanism, where the second portion **50** may advance further into the cavity of the first portion **48**.

An injecting needle is firmly implanted within the second portion **50** with both ends extending beyond the second portion **50**. A casing **54** surrounds the second portion **50** and prevents the second portion from advancing into the first portion **48** prematurely. A spacer **56** is attached to the second portion **50**, and coupled thereto is additionally a cap **58** with an antiseptic applicator **57** attached thereto. Applicator **57** includes a sponge and a protective foil **59**, such as described in more detail in conjunction with FIG. **1**.

Referring to FIG. **9**, apparatus **77** has been arranged for immediate use. The casing **54** has been removed so that the second portion **50** may be twisted and advanced further into the cavity of the first portion **48**. In doing so, the needle **52** pierces the stopper element **82** and reaches the hollow body **78** of the syringe. Apparatus **77** may now be used like a standard syringe **76** for injecting any liquid to be drawn into the hollow body **78** of the syringe **76** through the needle **52**. In addition, the spacer **56** effectively determines the depth of needle penetration into a patient's skin.

Another apparatus **77'** that is constructed in accordance with the invention is shown in FIGS. **10** and **11**. Apparatus **77'** is comprised of a syringe-like element **76** like that of apparatus **77** shown in FIGS. **8** and **9**, and the seat member **65** shown in FIGS. **6** and **7**. Hereinafter, like numerals refer to like elements in FIGS. **6–9** to more clearly illustrate the structure and function of apparatus **77'**.

Apparatus **77'** includes a syringe-like element **76** with a hollow body **78** and a plunger **80**. Plunger **80** may slidably

advance and retreat within the hollow body **78** to effectively decrease and increase the effective capacity of the hollow body **78**. A stopper element **82** forms another liquid proof barrier at the other end of the hollow body **78**.

Coupled to the syringe element **76** is a seat member **65**. Seat member **65** includes a first portion **64** and a second portion **66**. A hollow needle **68** is firmly implanted longitudinally within the second seat member portion **66**, and extends substantially beyond the second portion **66** at both ends. The second portion **66** is structurally divided into a short and a long end by a stop **70**. The short end may also be constructed to have a substantially larger diameter than the long end. When not in use, as shown in FIG. **10**, the short end of the second portion **66** is inserted into a cavity of the first portion **64**. Arranged in this fashion, the needle **68** cannot reach and pierce the stopper element **82** of the syringe-like element **76**. Additionally, a spacer **72** is arranged between the second portion **66** and the first portion **64**, and a cap **74** is detachably coupled to the long end of the second portion **66**. Preferably, the cap **74** includes an antiseptic applicator **73** which may consist of a sponge saturated with an antiseptic solution and a protective foil **75**.

Referring presently to FIG. **11**, the apparatus **77'** has been arranged for immediate use. Similar to the apparatus shown in FIGS. **6** and **7**, the second portion **66** has been rearranged so that the long end is inserted into the cavity of the first portion **64**. Arranged in this manner, the needle **68** is allowed to penetrate the stopper element **82** and reach into the cavity formed by the hollow body **78**. Similarly, the spacer **72**, still attached to the short end of second portion **66**, provides a means for determining the needle penetration depth into the skin.

Referring presently to FIG. **12**, an alternate embodiment of a twist-lock mechanism is shown. Storage chamber **92** is coupled to a rigid seat member **90** incorporating the twist-lock mechanism therein. The rigid seat member **90** is constructed of two portions **94** and **96**, where portion **96** is adapted for advancing into the hollow outer portion **94**. A hollow needle **97** is tightly gripped by portion **96** and is aligned longitudinally with respect to the rigid seat member **90**. The outer portion of seat member **90** further defines a generally Z-shaped groove **98** formed on its inner surface. The Z-shaped groove **98** generally has three portions, a longer and generally longitudinal portion substantially aligned with respect to the needle, and two shorter circumferential portions extending generally perpendicularly from the respective ends of the longer portion. As shown in FIG. **12**, the shorter portions extend from the longer portion in two opposing directions. On the portion **96** a protrusion or node **100** is formed, which is adapted for mating with Z-shaped groove **98** and progressing along it as it is manipulated to advance needle **97** toward storage chamber **92**.

Therefore, to advance the needle **97** toward the storage chamber **92**, the portion **96** is first twisted or rotated with respect to portion **94** so that node **100** proceeds along the lower shorter portion of the z-shaped groove **98**. Then portion **96** can be pushed toward storage chamber **92** where it advances further into portion **94** so that the needle **97** pierces the storage chamber **92**. At this time, node **100** is proceeding along the longer portion of the groove **98**. Finally, portion **96** is twisted or rotated so that node **100** comes to rest in the recess formed by the upper shorter portion of the groove **98**, effectively locking portion **96** thereby preventing any longitudinally movement thereof.

FIGS. **13** and **14** show examples of alternative embodiments of the groove and node combination unfurled from the concave inner surface of rigid seat member portion **94**. Groove **98'** as shown in FIG. **13** further includes a recess **102** at the one of one of the shorter portions. The node **100'** can come to rest in the recess **102** after it reaches the end of groove **98'** closest to the storage chamber **92**. This embodiment further ensures that portion **96** is "locked" in the second position after the needle **97** has pierced the storage chamber **92**. FIG. **13** illustrates a groove **98"** oriented diagonally with respect to the needle **96**. The shorter portions thereof are also arranged at an incline as shown. Node **100"** is accordingly similarly oriented to ensure conformity with groove **98"**.

It is evident that the use of twist-lock mechanisms such as shown in FIGS. **4**, **5** and **12–14** facilitates and simplifies the preparation of the apparatus for use. A minimal of rotation of seat member portion **96** is required to advance the needle **97** to the storage chamber **92** and to lock it in place. Also shown in FIG. **12** is a spacing device **104** which may be advantageous used to vary the depth of needle penetration, as described above.

The use of the various embodiments of the anesthetizing apparatus, as shown in the accompanying drawings and described above, is best described in relation to its application in the field of medicine. More specifically, the various embodiments of the apparatus are used to numb a small area of skin by raising a wheal thereon prior to inserting an I.V. needle and catheter or for injecting various medications or medicated solutions for diagnostic proposes including allergy testing. The administration of the anesthetizing apparatus of the various embodiments is substantially the same. Referring to FIG. **15**, the apparatus is held substantially perpendicularly to a patient's skin surface over the intended site of I.V. injection. The apparatus is then lowered onto the skin surface until the fine-gauge needle **130** penetrates the skin. Penetration is stopped by the spacer **128** so that the depth of penetration is determined by the spacer and the length of the needle **130**. As shown, differing needle lengths **130–134** may be provided to deposit the anesthetizing agent in the epidermis layer, the dermis layer, the tela subcutanea layer, or between any of these layers. Since the skin thickness generally range between 0.5 to six millimeters, the needle length may vary accordingly dependent on the desired site of the I.V. The anesthetizing agent is then introduced into the skin simultaneously by applying pressure to the storage chamber or plunger. The apparatus is easily operable with one hand and is disposable after use.

In contrast, when conventional needles and syringes are used to numb a site, many steps are involved. A needle of typical length and size is first selected and installed on the syringe and used to extract the anesthetic agent from a bottle or vial. The needle must then be removed and exchanged for a finer needle. Because of the longer length of conventional needles, they must be inserted at an acute angle or generally tangentially with respect to the skin in order to deposit the solution in the skin layers to raise a skin wheal. Accordingly skill and experience are thus required to judge the depth of needle penetration and stop advancing the needle into the skin for best results. The use of conventional devices to raise a skin wheal also requires needless expenditures of time and expense.

Accordingly, the pain and suffering usually associated with an I.V. needle or diagnostic testing is reduced or eliminated, lessening the emotional and physical burden on the patient. The apparatus is compact and self contained. The apparatus requires little preparation prior to use and is disposable after use.

While the embodiments of the invention have been described in detail, it will be understood that many changes and modifications can be made thereto without departing from the spirit or scope of the invention. Other aspects, objects, and advantages of this invention can be obtained from a study of the drawings, the disclosure, and the appended claims.

What is claimed is:

1. A method for raising a skin wheal in preparation for an intravenous injection, comprising the steps of:

perpendicularly inserting substantially the full length of a fine-gauge needle having a length approximating the intended depth of skin penetration into the skin;

injecting an anesthetic solution; and

removing the needle.

2. The method, as set forth in claim **1**, wherein the step of inserting the needle into the skin includes the step of inserting the needle to inject the anesthetic solution to the epidermis and dermis layers of the skin.

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SEVENTEENTH EDITION

THE MERCK MANUAL

OF

DIAGNOSIS AND THERAPY

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FOREWORD

With this edition, *The Merck Manual* celebrates its 100th birthday. When the editors of the 1st Edition produced their 192-page compendium, they could not have realized the extent to which medical knowledge would explode over the next century. *The Merck Manual* now fills 2,655 pages and covers countless diseases that were not known 100 years ago. A brief review of medical practice as reflected in *The Merck Manual* during the past century follows on page vii.

Although the knowledge of medicine has grown, the goal of *The Merck Manual* has not changed—To provide useful clinical information to practicing physicians, medical students, interns, residents, nurses, pharmacists, and other health care professionals in a concise, complete, and accurate manner. *The Merck Manual* continues to cover all the subjects expected in a textbook of internal medicine as well as detailed information on pediatrics, psychiatry, obstetrics, gynecology, dermatology, pharmacology, ophthalmology, otolaryngology, and a number of special subjects. *The Merck Manual* quickly provides information that helps practitioners achieve optimal care. The more specialized the practice of medicine becomes, the more important such information becomes. Specialists as well as generalists must at some time quickly access information about other specialties.

The 17th edition of *The Merck Manual* is the culmination of an arduous but rewarding 7-year enterprise. Every topic has been updated, and many have been completely rewritten. Topics new to this edition include hand disorders, prion diseases, death and dying, probabilities in clinical medicine, multiple chemical sensitivity, chronic fatigue syndrome, rehabilitation, smoking cessation, and drug therapy in the elderly, among others. The members of the Editorial Board, special consultants, and contributing authors are listed on the following pages with their affiliations. They deserve a degree of gratitude that cannot be adequately expressed here, but we know they will feel sufficiently rewarded if their efforts serve your needs.

Because of the extensive subject matter covered and a successful tradition developed through trials of successes and failures, *The Merck Manual* has some unique characteristics. We urge readers to spend a few minutes reviewing the Guide for Readers (p. xii), the Table of Contents at the beginning of each section (indicated by a thumb tab), and the Index (p. 2657). Subject headings within each section, internal headings within a subject discussion, and boldfaced terms in the text form an outline intended to help with use of the text.

We hope this edition of *The Merck Manual* will serve as an aid to you, our readers, compatible with your needs and worthy of frequent use. Suggestions for improvements will be warmly welcomed and carefully considered.

MARK H. BEERS, M.D., and ROBERT BERKOW, M.D., *Editors*

SECTION 22

CLINICAL PHARMACOLOGY

298 / DRUG INPUT AND DISPOSITION

Drugs are almost always compounds foreign to the body. As such, they, unlike endogenous substances, are not continually being formed and eliminated. Drug absorption, bioavailability, distribution, and elimination are therefore determinants of onset, duration, and intensity of drug effect.

ABSORPTION

Process of drug movement from the administration site to the systemic circulation.

Drug absorption is determined by physicochemical properties of drugs, their formulations, and routes of administration. **Drug products**—the actual dosage forms (eg, tablets, capsules, solutions), consisting of the drug plus other ingredients—are formulated to be administered by various routes, including oral, buccal, sublingual, rectal, parenteral, topical, and inhalational. A prerequisite to absorption is drug dissolution. Solid drug products (eg, tablets) disintegrate and deaggregate, but absorption can occur only after drugs enter solution.

Transport Across Cell Membranes

When given by most routes (excluding IV), a drug must traverse several semipermeable cell membranes before reaching the systemic circulation. These membranes are biologic barriers that selectively inhibit the passage of drug molecules and are composed primarily of a bimolecular lipid matrix, containing mostly cholesterol and phospholipids. The lipids provide stability to the membrane and determine its permeability characteristics. Globular proteins of various sizes and composition are embedded in the matrix; they are involved in transport and function as receptors for cellular regulation. Drugs may cross a biologic barrier by passive diffusion, facilitated passive diffusion, active transport, or pinocytosis.

Passive diffusion: In this process, transport across a cell membrane depends on the concentration gradient of the solute. Most drug molecules are transported across a membrane by simple diffusion from a region of high concentration (eg, GI fluids) to one of low concentration (eg, blood). Because drug molecules are rapidly removed by the systemic circulation and distributed into a large volume of body fluids and tissues, drug concentration in blood is initially low compared with that at the administration site, producing a large gradient. The diffusion rate is directly proportional to the gradient but also depends on the molecule's lipid solubility, degree of ionization, and size and on the area of the absorptive surface. Because the cell membrane is lipoid, lipid-soluble drugs diffuse more rapidly than relatively lipid-insoluble drugs. Small molecules tend to penetrate membranes more rapidly than large ones.

Most drugs are weak organic acids or bases, existing in un-ionized and ionized forms in an aqueous environment. The un-ionized form is usually lipid soluble and diffuses readily across cell membranes. The ionized form cannot penetrate the cell membrane easily because of its low lipid solubility and high electrical resistance, resulting from its charge and the charged groups on the cell membrane surface. Thus, drug penetration may be attributed mostly to the un-ionized form. Distribution of an ionizable drug across a membrane at equilibrium is determined by the drug's pK_a (the pH at which concentrations of un-ionized and ionized forms of the drug are equal) and the pH gradient, when present. For a weak acid, the higher the pH, the lower the ratio of un-ionized to ionized forms. In plasma (pH, 7.4), the ratio of un-ionized to ionized forms for a weak acid (eg, with a pK_a of 4.4) is 1:1000; in gastric fluid (pH, 1.4), the ratio is reversed (1000:1). When the weak acid is given orally, the concentration gradient for un-ionized drug between stomach and plasma tends to be large, favoring diffusion through the gastric mucosa. At equilibrium, the concentrations of un-ionized drug in the stomach and in the plasma are equal because only un-ionized drug can penetrate the membranes; the concentration of ionized drug in the plasma would then be about 1000 times greater than that in the stomach. For a weak base with a pK_a of 4.4, the outcome is reversed. Thus theoretically, weakly acidic drugs (eg, aspirin) are more readily absorbed from an acid medium (stomach) than are weak bases (eg, quinidine). However, whether a drug is acidic or basic, most of its absorption occurs

in the small intestine (see Oral Administration, below).

Facilitated passive diffusion: For certain molecules (eg, glucose), the rate of membrane penetration is greater than expected from their low lipid solubility. One theory is that a carrier component combines reversibly with the substrate molecule at the cell membrane exterior, and the carrier-substrate complex diffuses rapidly across the membrane, releasing the substrate at the interior surface. Carrier-mediated diffusion is characterized by selectivity and saturability: The carrier transports only substrates with a relatively specific molecular configuration, and the process is limited by the availability of carriers. The process does not require energy expenditure, and transport against a concentration gradient does not occur.

Active transport: This process is characterized by selectivity and saturability and requires energy expenditure by the cell. Substrates may accumulate intracellularly against a concentration gradient. Active transport appears to be limited to drugs structurally similar to endogenous substances. These drugs are usually absorbed from sites in the small intestine. Active transport processes have been identified for various ions, vitamins, sugars, and amino acids.

Pinocytosis: Fluid or particles are engulfed by a cell. The cell membrane invaginates, encloses the fluid or particles, then fuses again, forming a vesicle that later detaches and moves to the cell interior. This mechanism also requires energy expenditure. Pinocytosis probably plays a minor role in drug transport, except for protein drugs.

Oral Administration

For oral administration, the most common route, absorption refers to the transport of drugs across membranes of the epithelial cells in the GI tract. Absorption after oral administration is confounded by differences in luminal pH along the GI tract, surface area per luminal volume, blood perfusion, the presence of bile and mucus, and the nature of epithelial membranes. Acids are absorbed faster in the intestine than in the stomach, apparently contradicting the hypothesis that un-ionized drug more readily crosses membranes. However, the apparent contradiction is explained by the larger surface area and greater permeability of the membranes in the small intestine.

The oral mucosa has a thin epithelium and a rich vascularity that favors absorption, but contact is usually too brief, even for drugs in solution, for appreciable absorption to occur. A drug placed between the gums and cheek (buccal administration) or under the tongue (sublingual administration) is retained longer so that absorption is more complete.

The stomach has a relatively large epithelial surface, but because it has a thick mucous layer and the time that the drug remains there is usually relatively short, absorption is limited. Absorption of virtually all drugs is faster from the small intestine than from the stomach. Therefore, gastric emptying is the rate-limiting step. Food, especially fatty foods, slows gastric emptying (and the rate of drug absorption), explaining why some drugs should be taken on an empty stomach when a rapid onset of action is desired. Food may enhance the extent of absorption for poorly soluble drugs (eg, griseofulvin), reduce it for drugs degraded in the stomach (eg, penicillin G), or have little or no effect. Drugs that affect gastric emptying (eg, parasympatholytic drugs) affect the absorption rate of other drugs.

The small intestine has the largest surface area for drug absorption in the GI tract. The intraluminal pH is 4 to 5 in the duodenum but becomes progressively more alkaline, approaching 8 in the lower ileum. GI microflora may inactivate certain drugs, reducing their absorption. Decreased blood flow (eg, in shock) may lower the concentration gradient across the intestinal mucosa and decrease absorption by passive diffusion. (Decreased peripheral blood flow also alters drug distribution and metabolism.)

Intestinal transit time can influence drug absorption, particularly for drugs that are absorbed by active transport (eg, B vitamins), that dissolve slowly (eg, griseofulvin), or that are too polar (ie, poorly lipid-soluble) to cross membranes readily (eg, many antibiotics). For such drugs, transit may be too rapid for absorption to be complete.

For controlled-release dosage forms, absorption may occur primarily in the large intestine, particularly when drug release continues for > 6 h, the time for transit to the large intestine.

Absorption from solution: A drug given orally in solution is subjected to numerous GI secretions and, to be absorbed, must sur-

vive encounters with low pH and potentially degrading enzymes. Usually, even if a drug is stable in the enteral environment, little of it remains to pass into the large intestine. Drugs with low lipophilicity (ie, low membrane permeability), such as aminoglycosides, are absorbed slowly from solution in the stomach and small intestine; for such drugs, absorption in the large intestine is expected to be even slower because the surface area is smaller. Consequently, these drugs are not candidates for controlled release.

Absorption from solid forms: Most drugs are given orally as tablets or capsules primarily for convenience, economy, stability, and patient acceptance. These products must disintegrate and dissolve before absorption can occur. Disintegration greatly increases the drug's surface area in contact with GI fluids, thereby promoting drug dissolution and absorption. Disintegrants and other excipients (eg, diluents, lubricants, surfactants, binders, dispersants) are often added during manufacture to facilitate these processes. Surfactants increase the dissolution rate by increasing the wetability, solubility, and dispersibility of the drug. Disintegration of solid forms may be retarded by excessive pressure applied during the tableting procedure or by special coatings applied to protect the tablet from the digestive processes of the gut. Hydrophobic lubricants (eg, magnesium stearate) may bind to the active drug and reduce its bioavailability.

Dissolution rate determines the availability of the drug for absorption. When slower than absorption, dissolution becomes the rate-limiting step. Overall absorption can be controlled by manipulating the formulation. For example, reducing the particle size increases the drug's surface area, thus increasing the rate and extent of GI absorption of a drug whose absorption is normally limited by slow dissolution. Dissolution rate is affected by whether the drug is in salt, crystal, or hydrate form. The Na salts of weak acids (eg, barbiturates, salicylates) dissolve faster than their corresponding free acids regardless of the pH of the medium. Certain drugs are polymorphic, existing in amorphous or various crystalline forms. Chloramphenicol palmitate has two forms, but only one sufficiently dissolves and is absorbed to be clinically useful. A hydrate is formed when one or more water molecules combine with a drug molecule in crystal form. The solubility of such a solvate may markedly differ from the nonsolvated form; eg, anhydrous ampicillin has a greater rate of dissolution and absorption than its corresponding trihydrate.

Parenteral Administration

Direct placement of a drug into the bloodstream (usually IV) ensures delivery of the dose to the systemic circulation. However, delivery of the entire dose is not ensured if a route requires movement through one or more biologic membranes to reach the systemic circulation (IM or sc injection). For protein drugs with a molecular mass > 20,000 g/mol, movement across capillary membranes is so slow that after IM or sc administration, most absorption occurs via the lymphatic system by default. In such cases, the delivery rate to systemic circulation is slow and often incomplete because of first-pass metabolism by proteolytic enzymes in the lymphatics.

Because capillaries tend to be highly porous, perfusion (blood flow/gram of tissue) greatly affects the absorption rate of small molecules. Thus, the injection site can markedly influence a drug's absorption rate; eg, the absorption rate of diazepam injected IM into a site with poor blood flow can be much slower than that after oral administration.

Absorption may be delayed or erratic when salts of poorly soluble acids and bases are injected IM. The parenteral form of phenytoin is a 40% propylene glycol solution of the Na salt with a pH of about 12. When the solution is injected IM, the propylene glycol is absorbed, and the tissue fluids, acting as a buffer, decrease the pH, shifting the equilibrium between the ionized and free acid forms of the drug. The poorly soluble free acid then precipitates. As a result, dissolution and absorption take 1 to 2 wk to occur.

Controlled-Release Forms

Controlled-release dosage forms are designed to reduce dosing frequency and to reduce fluctuation in plasma drug concentration, providing a more uniform therapeutic effect. Less frequent dosing is more convenient and may improve patient compliance. These dosage forms are suitable for drugs that otherwise require frequent dosing because elimination half-life and duration of effect are short.

Oral controlled-release forms are often designed to maintain therapeutic drug concentrations for ≥ 12 h. The absorption rate can be controlled by coating drug particles with wax or other water-insoluble material, by embedding the drug in a matrix from which it is released slowly during transit through the GI tract, or by complexing the drug with ion-exchange resins.

Transdermal controlled-release forms are designed to provide drug release for extended periods; eg, clonidine diffusion through a membrane provides controlled drug delivery for 1 wk, and nitroglycerin-impregnated polymer bonded to an adhesive bandage provides controlled drug delivery for 24 h. Drugs for transdermal delivery must have suitable skin penetration characteristics and high potency because the penetration rate and area of application are limited.

Many nonintravenous parenteral preparations are formulated to sustain blood levels. For antimicrobials, relatively insoluble salts (eg, penicillin G benzathine) injected IM provide therapeutic concentrations for extended periods. For others, suspensions or solutions in nonaqueous vehicles (eg, insulin injected in crystalline suspensions) are formulated. Amorphous insulin, with a high surface area for dissolution, has a rapid onset and short duration of action.

BIOAVAILABILITY

Extent to which—and sometimes rate at which—the active moiety (drug or metabolite) enters systemic circulation, thereby gaining access to the site of action.

The physicochemical properties of a drug govern its absorptive potential, but the properties of the dosage form (which partly depend on its design and manufacture) can largely determine drug bioavailability. Differences in bioavailability among formulations of a given drug can have clinical significance. Thus, the concept of equivalence among drug products is important in making clinical decisions. **Chemical equivalence** refers to drug products that contain the same compound in the same amount and that meet current official standards; however, inactive ingredients in drug products may differ. **Bioequivalence** refers to chemical equivalents that, when administered to the same person in the same dosage regimen, result in equivalent concentrations of drug in blood and tissues. **Therapeutic equivalence** refers to drug products that, when administered to the same person in the same dosage regimen, provide essentially the same therapeutic effect or toxicity. Bioequivalent products are expected to be therapeutically equivalent.

Therapeutic problems (eg, toxicity, lack of efficacy) are encountered most frequently during long-term therapy when a patient who is stabilized on one formulation is given a nonequivalent substitute (as for digoxin or phenytoin).

Sometimes therapeutic equivalence may be achieved despite differences in bioavailability. For example, the therapeutic index (ratio of the maximum tolerated dose to the minimum effective dose) of penicillin is so wide that moderate blood concentration differences due to bioavailability differences in penicillin products may not affect therapeutic efficacy or safety. In contrast, bioavailability differences are important for a drug with a relatively narrow therapeutic index.

The physiologic characteristics and comorbidities of the patient also affect bioavailability.

Absorption rate is important because even when a drug is absorbed completely, it may be absorbed too slowly to produce a therapeutic blood level quickly enough or so rapidly that toxicity results from high drug concentrations after each dose.

Causes of Low Bioavailability

When a drug rapidly dissolves and readily crosses membranes, absorption tends to be complete, but absorption of orally administered drugs is not always complete. Before reaching the vena cava, a drug must move down the GI tract and pass through the gut wall and liver, common sites of drug metabolism (see Ch. 43); thus, a drug may be metabolized (first-pass metabolism) before it can be measured in the systemic circulation. Many drugs have low oral bioavailability because of extensive first-pass metabolism. For such drugs (eg, isoproterenol, norepinephrine, testosterone), extraction in these tissues is so extensive that bioavailability is virtually zero. For drugs with an active metabolite, the therapeutic consequence of first-pass metabolism depends on the contri-

butions of the drug and the metabolite to the desired and undesired effects.

Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. More factors can affect bioavailability when absorption is slow or incomplete than when it is rapid and complete, so slow or incomplete absorption often leads to variable therapeutic responses.

Insufficient time in the GI tract is a common cause of low bioavailability. Ingested drug is exposed to the entire GI tract for no more than 1 to 2 days and to the small intestine for only 2 to 4 h. If the drug does not dissolve readily or cannot penetrate the epithelial membrane (eg, if it is highly ionized and polar), time at the absorption site may be insufficient. In such cases, bioavailability tends to be highly variable as well as low. Age, sex, activity, genetic phenotype, stress, disease (eg, achlorhydria, malabsorption syndromes), or previous GI surgery can affect drug bioavailability.

Reactions that compete with absorption can reduce bioavailability. They include complex formation (eg, between tetracycline and polyvalent metal ions), hydrolysis by gastric acid or digestive enzymes (eg, penicillin and chloramphenicol palmitate hydrolysis), conjugation in the gut wall (eg, sulfoconjugation of isoproterenol), adsorption to other drugs (eg, digoxin and cholestyramine), and metabolism by luminal microflora.

Assessment of Bioavailability

Assessment of bioavailability from plasma concentration–time data usually involves determining the maximum (peak) plasma drug concentration, the time at which maximum plasma drug concentration occurs (peak time), and the area under the plasma concentration–time curve (AUC—see FIG. 298–1). The plasma drug concentration increases with the extent of absorption; the peak is reached when the drug elimination rate equals absorption rate. Bioavailability determinations based on the peak plasma concentration can be misleading, because drug elimination begins as soon as the drug enters the bloodstream. The most widely used general index of absorption rate is peak time; the slower the absorption, the later the peak time. However, peak time is often not a good statistical measure because it is a discrete value that depends on frequency of blood sampling and, in the case of relatively flat concentrations near the peak, on assay reproducibility.

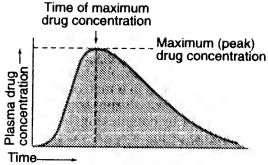

FIG. 298–1. Representative plasma concentration–time relationship after a single oral dose of a hypothetical drug. Area under the plasma concentration–time curve is indicated by shading.

AUC is the most reliable measure of bioavailability. It is directly proportional to the total amount of unchanged drug that reaches the systemic circulation. For an accurate measurement, blood must be sampled frequently over a long enough time to observe virtually complete drug elimination. Drug products may be considered bioequivalent in extent and rate of absorption if their plasma-level curves are essentially superimposable. Drug products that have similar AUCs but differently shaped plasma-level curves are equivalent in extent but differ in their absorption rate–time profiles.

Single vs. multiple doses: Bioavailability may be assessed after single or repetitive (multiple) dosing. More information about rate of absorption is available after a single dose than after multiple dosing. However, multiple dosing more closely represents the usual clinical situation, and plasma concentrations are usually higher than those after a single dose, facilitating data analysis. After multiple dosing at a fixed-dosing interval for four or five elimination half-lives, the blood drug concentration should be at steady state (the amount absorbed equals the amount eliminated within each dosing interval). The extent of absorption can then be analyzed by measuring the AUC during a dosing interval. Measuring the AUC over 24 h is probably preferable because of circadian variations in physiologic functions and because of possible variations in dosing intervals and absorption rates during a day.

For drugs excreted primarily unchanged in urine, bioavailability can be estimated by

measuring the total amount of drug excreted after a single dose. Ideally, urine is collected over a period of 7 to 10 elimination half-lives for complete urinary recovery of the absorbed drug. Bioavailability may also be assessed after multiple dosing by measuring unchanged drug recovered from urine over 24 h under steady-state conditions.

DISTRIBUTION

After a drug enters the systemic circulation, it is distributed to the body's tissues. Distribution is generally uneven because of differences in blood perfusion, tissue binding, regional pH, and permeability of cell membranes.

The entry rate of a drug into a tissue depends on the rate of blood flow to the tissue, on tissue mass, and on partition characteristics between blood and tissue. Distribution equilibrium (when entry and exit rates are the same) between blood and tissue is reached more rapidly in richly vascularized areas than in poorly perfused areas, unless diffusion across membrane barriers is the rate-limiting step. After equilibrium is attained, drug concentrations (bound and unbound—see below) in tissues and in extracellular fluids are reflected by the plasma concentration. Metabolism and excretion occur simultaneously with distribution, making the process dynamic and complex (see also Ch. 299).

Apparent Volume of Distribution

The volume of fluid into which a drug appears to be distributed or diluted is called the apparent volume of distribution (the fluid volume required to contain the drug in the body at the same concentration as in plasma). This parameter provides a reference for the plasma concentration expected for a given dose and for the dose required to produce a given concentration. However, it provides little information about the specific pattern of distribution. Each drug is uniquely distributed in the body. Some drugs go into fat, others remain in the ECF, and still others are bound avidly to specific tissues, commonly liver or kidney.

Many acidic drugs (eg, warfarin, salicylic acid) are highly protein-bound and thus have a small apparent volume of distribution. Many basic drugs (eg, amphetamine, meperidine) are avidly taken up by tissues and thus have an apparent volume of distribution larger than the volume of the entire body.

Binding

The extent of drug distribution into tissues depends on the extent of plasma protein and tissue binding.

Plasma protein binding: Drugs are transported in the bloodstream partly in solution as free (unbound) drug and partly bound to blood components (eg, plasma proteins, blood cells). The ratio of bound to unbound drug in plasma is mainly determined by the reversible interaction between a drug and the plasma protein to which it binds, as governed by the law of mass action. Many plasma proteins can interact with drugs. Albumin, α_1-acid glycoprotein, and lipoproteins are most important. Acidic drugs are generally bound more extensively to albumin, and basic drugs to α_1-acid glycoprotein and/or lipoproteins (see TABLE 298–1).

Only unbound drug is thought to be available for passive diffusion to extravascular or tissue sites where pharmacologic effects occur. Therefore, the unbound drug concentration may be more closely related to drug concentration at the active site and to drug effects, often making the fraction unbound (ratio of unbound to total concentrations) a more useful parameter than the fraction bound. Plasma protein binding influences distribution and the apparent relationship between pharmacologic activity and total

TABLE 298–1. EXTENT OF BINDING IN PLASMA FOR SELECTED DRUGS

Drug	% Bound	% Unbound
Warfarin	99.5	0.5
Diazepam	99	1
Furosemide	96	4
Dicloxacillin	94	6
Propranolol*	93	7
Phenytoin	89	11
Quinidine*	71	29
Lidocaine*	51	49
Digoxin	25	75
Gentamicin	3	97
Atenolol	~0	~100

*Significant binding to α_1-acid glycoprotein and/or lipoproteins.

plasma drug concentration. At high drug concentrations, the amount of bound drug approaches an upper limit depending on the number of available binding sites, resulting in **saturability.** Saturability is the basis of displacement interactions among drugs (see DRUG INTERACTIONS in Ch. 301).

Tissue binding: Drugs bind to many substances other than proteins. Binding may be very specific, as when chloroquine binds with nucleic acids. Binding usually occurs when a drug associates with a macromolecule in an aqueous environment but may occur when a drug is partitioned into body fat. Because fat is poorly perfused, equilibration time is long, especially if the drug has a high affinity for fat.

Drug reservoir: Accumulation of drugs in tissues or body compartments can prolong the sojourn of drug in plasma and drug action because the tissues release stored drug as the plasma concentration declines. Location of the active site and relative differences in tissue distribution can also be important. For the anesthetic thiopental, storage in tissue reservoirs initially shortens the drug effect but after repeated administration prolongs it. Thiopental is highly lipid soluble and rapidly distributes to the brain after a single IV injection. After a single dose, thiopental concentration in the brain increases for a few minutes, then declines parallel with the plasma concentration. Anesthesia ends rapidly as the drug redistributes to more slowly perfused tissues. However, if plasma concentration is monitored long enough, a third phase of distribution, in which the drug is slowly released from fat, can be distinguished. With continued administration of thiopental, large amounts may be stored in fat, resulting in prolongation of anesthetic plasma concentrations.

Some drugs accumulate, producing higher concentrations in cells than in ECF, most commonly because they bind with protein, phospholipids, or nucleic acids. Antimalarial drugs (eg, chloroquine) produce concentrations within WBCs and liver cells thousands of times higher than those in plasma. The stored drug is in equilibrium with drug in plasma and moves into plasma as the drug is eliminated from the body.

Blood-Brain Barrier

Drugs reach the CNS via brain capillaries and via CSF. Although the brain receives about 1/6 of cardiac output, distribution of drugs to brain tissue is restricted. Some lipid-soluble drugs (eg, thiopental) enter the brain and exert their pharmacologic effects rapidly, but many drugs, particularly the more water-soluble drugs, enter the brain slowly. The endothelial cells of the brain capillaries, which appear to be more tightly joined to one another than are those of other capillaries, contribute to the slow diffusion of water-soluble drugs. Another barrier to water-soluble drugs is the glial connective tissue cells (astrocytes), which form an astrocytic sheath close to the basement membrane of the capillary endothelium. The capillary endothelium and the astrocytic sheath form the blood-brain barrier. Because the capillary wall rather than the parenchymal cell forms the barrier, the brain's permeability characteristics differ from those of other tissues. Thus, polar compounds cannot enter the brain but can enter the interstitial fluids of most other tissues. The observation that polar dyes enter most tissues but not the CNS led to the concept of the blood-brain barrier.

Drugs may enter ventricular CSF directly via the choroid plexus, entering brain tissue by passive diffusion from CSF. Also in the choroid plexus, organic acids (eg, penicillin) are actively transported from CSF to blood.

The drug penetration rate into the CSF or into other tissue cells is determined mainly by the extent of protein binding, the degree of ionization, and the lipid-water partition coefficient of the drug. The penetration rate into the brain is slow for highly protein-bound drugs and can be so slow for the ionized form of weak acids and bases as to be virtually nonexistent.

Because the CNS is so well perfused, permeability is generally the major determinant of the drug distribution rate. However, for the interstitial fluids of most tissues, perfusion is a major determinant. For poorly perfused tissues (eg, muscle, fat), distribution is very slow, especially if the tissue has a high affinity for the drug.

ELIMINATION

Sum of the processes of drug loss (metabolism and excretion) from the body.

METABOLISM

The liver is the principal site of drug metabolism (chemical alteration) in the body.

TABLE 298–2. SELECTED DRUGS WITH THERAPEUTICALLY IMPORTANT METABOLITES

Drug	Metabolite
Acetohexamide	Hydroxyhexamide
Amitriptyline	Nortriptyline
Aspirin*	Salicylic acid
Chloral hydrate*	Trichloroethanol
Chlordiazepoxide	Desmethylchlordiazepoxide
Codeine	Morphine
Diazepam	Desmethyldiazepam
Flurazepam	Desethylflurazepam
Glutethimide	4-Hydroxyglutethimide
Imipramine	Desipramine
Lidocaine	Desethyllidocaine
Meperidine	Normeperidine
Phenacetin*	Acetaminophen
Phenylbutazone	Oxyphenbutazone
Prednisone*	Prednisolone
Primidone*	Phenobarbital
Procainamide	*N*-acetylprocainamide
Propranolol	4-Hydroxypropranolol

*Pro-drugs; metabolites are primarily responsible for their therapeutic effects.

Some metabolites are pharmacologically active (see TABLE 298–2). An inactive substance that has an active metabolite is called a pro-drug, especially if designed to deliver the active moiety more effectively.

Pathways of Metabolism

Drug metabolism involves a wide range of chemical reactions, including oxidation, reduction, hydrolysis, hydration, conjugation, condensation, and isomerization. The enzymes involved are present in many tissues but generally are more concentrated in the liver. For many drugs, metabolism occurs in two apparent phases. Phase I reactions involve the formation of a new or modified functional group or a cleavage (oxidation, reduction, hydrolysis); these are nonsynthetic reactions. Phase II reactions involve conjugation with an endogenous compound (eg, glucuronic acid, sulfate, glycine) and are therefore synthetic reactions. Metabolites formed in synthetic reactions are more polar and more readily excreted by the kidneys (in urine) and the liver (in bile) than those formed in nonsynthetic reactions. Some drugs undergo either phase I or phase II reactions; thus, phase numbers reflect functional rather than sequential classification.

Cytochrome P-450: The most important enzyme system of phase I metabolism is cytochrome P-450, a microsomal superfamily of isoenzymes that transfer electrons and thereby catalyze the oxidation of many drugs. The electrons are supplied by NADPH–cytochrome P-450 reductase, a flavoprotein that transfers electrons from NADPH (the reduced form of nicotinamide-adenine dinucleotide phosphate) to cytochrome P-450. Cytochrome P-450 enzymes are grouped into 14 mammalian gene families that share sequence identity and 17 subfamilies. They are designated by a root symbol CYP, followed by an Arabic number for family, a letter for subfamily, and another Arabic number for the specific gene. Enzymes in the 1A, 2B, 2C, 2D, and 3A subfamilies are most important in mammalian metabolism; CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are important in human metabolism. The specificity of the enzymes helps explain many drug interactions. Examples of drugs that interact with specific cytochrome P-450 enzymes are listed in TABLE 298–3 (see also DRUG INTERACTIONS in Ch. 301). Genetic differences among patients may alter response.

Conjugation: Glucuronidation, the most common phase II reaction, is the only one that occurs in the liver microsomal enzyme system. Glucuronides are secreted in bile and eliminated in urine. Chloramphenicol, meprobamate, and morphine are metabolized this way. Amino acid conjugation with glutamine or glycine produces conjugates (eg, salicyluric acid formed from salicylic acid and glycine) that are readily excreted in urine but are not extensively secreted in bile. Acetylation is the primary metabolic pathway for sulfonamides. Hydralazine, isoniazid, and procainamide are also acetylated. Sulfoconjugation is the reaction between phenolic or alcoholic groups and inorganic sulfate, which is partially derived from sulfur-containing amino acids (eg, cysteine). The sulfate esters formed are polar and readily excreted in urine. Drugs that form sulfate conjugates include acetaminophen, estradiol, methyldopa, minoxidil, and thyroxine. Methylation is a major metabolic pathway for inactivation of some catecholamines. Niacinamide and thiouracil are also methylated.

TABLE 298–3. SOME SUBSTANCES THAT INTERACT WITH CYTOCHROME P-450 ENZYMES

Enzyme	Substrates	Inhibitors	Inducers
CYP1A2	Acetaminophen Estradiol Theophylline Verapamil Warfarin	Furafylline	Charcoal-broiled beef Cigarette smoke
CYP2C9	Diclofenac Phenytoin Piroxicam Tetrahydrocannabinol Tolbutamide	Sulfaphenazole Sulfinpyrazone	Rifampin
CYP2C19	Diazepam Hexobarbital Omeprazole Pentamidine Propranolol	Tranylcypromine	Rifampin
CYP2D6	Debrisoquin Desipramine Encainide Mexiletine Nortriptyline	Fluoxetine Quinidine	None known
CYP3A4	Amiodarone Lovastatin Nifedipine Tamoxifen Terfenadine	Ketoconazole Troleandomycin	Carbamazepine Phenobarbital

Age-Related Changes

Because newborns have partially developed liver microsomal enzyme systems, they have difficulty metabolizing many drugs (eg, hexobarbital, phenacetin, amphetamine, chlorpromazine). In newborns, slower conversion to glucuronide can have serious effects. For example, equivalent mg/kg doses of chloramphenicol that are well tolerated by older patients can result in the gray baby syndrome and in prolonged elevated blood levels of chloramphenicol.

Elderly patients often have a reduced ability to metabolize drugs. The reduction varies depending on the drug and is not as severe as that in newborns (see Ch. 304).

Individual Variation

Because of individual variation (see also VARIABILITY IN PARAMETER VALUES in Ch. 299), predicting the clinical response to a given dose of a drug is difficult. Some patients metabolize a drug so rapidly that therapeutically effective blood and tissue concentrations are not achieved; in others, metabolism may be so slow that usual doses produce toxic effects. For example, plasma phenytoin concentrations at steady state vary from 2.5 to > 40 mg/L (10 to > 160 μmol/L) in different patients given a daily dose of 300 mg. Some variation is due to differences in the amount of the key enzyme, CYP2C9, available in the liver and to differences in the affinity of the enzyme for the drug. Genetic factors play a major role in determining these differences. Concurrent diseases (particularly chronic liver disease) and drug interactions (especially those involving induction or inhibition of metabolism) also contribute.

Capacity Limitation

For almost any drug, the rate of metabolism of any enzyme in any given pathway

reaches an upper limit (capacity limitation). At therapeutic concentrations, usually only a small fraction of the enzyme sites are occupied, and the rate of metabolism increases with drug concentration. Occasionally, when most of the enzyme sites are occupied, the rate of metabolism does not increase in proportion to drug concentration. The result is capacity-limited metabolism. Phenytoin and alcohol have this type of metabolism, which helps explain the interpatient variability in phenytoin concentrations after a fixed daily dose of 300 mg.

EXCRETION

Process by which a drug or a metabolite is eliminated from the body without further chemical change.

The kidneys, which excrete water-soluble substances, are the major organs of excretion. The biliary system contributes to excretion to the degree that drug is not reabsorbed from the GI tract. Generally, the contribution of intestine, saliva, sweat, breast milk, and lungs to excretion is small, except for exhalation of volatile anesthetics. Although excretion via breast milk may not be important to the mother, it may be to the suckling infant (see DRUGS IN LACTATING MOTHERS in Ch. 256).

Renal Excretion

Glomerular filtration and tubular reabsorption: About 1/5 of the plasma reaching the glomerulus is filtered through pores in the glomerular endothelium; the remainder passes through the efferent arterioles surrounding the renal tubules. Drugs bound to plasma proteins are not filtered; only unbound drug is contained in the filtrate. The principles of transmembrane passage govern renal tubular reabsorption of drugs. Polar compounds and ions cannot diffuse back into the circulation and are excreted unless a specific transport mechanism for their reabsorption exists (eg, as for glucose, ascorbic acid, and B vitamins).

Effects of urine pH: The glomerular filtrate that enters the proximal tubule has the same pH as plasma, but the pH of voided urine varies from 4.5 to 8.0. This variation in pH may markedly affect the rate of drug excretion. Because un-ionized forms of nonpolar weak acids and weak bases tend to be reabsorbed readily from tubular fluids, acidification of urine increases reabsorption (ie, decreases excretion) of weak acids and decreases reabsorption (ie, increases excretion) of weak bases. The opposite occurs after alkalinization of urine.

In some cases of overdose, these principles may be applied to enhance the excretion of weak acids or bases. For example, alkalinization of urine increases the excretion of the weak acids phenobarbital and aspirin, and acidification may accelerate the excretion of bases, such as methamphetamine. The extent to which changes in urinary pH alter the rate of drug elimination depends on the contribution of the renal route to total elimination as well as on the polarity of the un-ionized form and the degree of ionization of the molecule.

Tubular secretion: Mechanisms for active tubular secretion in the proximal tubule are important in the elimination of many drugs (eg, penicillin, mecamylamine, salicylic acid). This energy-dependent process may be blocked by metabolic inhibitors. When drug concentration is high, an upper limit for secretory transport can be reached; each substance has a characteristic maximum secretion rate (transport maximum).

Anions and cations are handled by separate transport mechanisms. Normally, the anion secretory system eliminates metabolites conjugated with glycine, sulfate, or glucuronic acid. Anionic compounds compete with one another for secretion. This competition can be used therapeutically; eg, probenecid blocks the normally rapid tubular secretion of penicillin, resulting in higher plasma penicillin concentrations for a longer time. Organic cations compete with each other but usually not with anions.

Age-related changes: With aging, renal drug excretion decreases (see PHARMACOKINETICS in Ch. 304 and TABLE 304–1).

Biliary Excretion

Drugs and their metabolites that are extensively excreted in bile are transported across the biliary epithelium against a concentration gradient, requiring active secretory transport. Secretory transport may approach an upper limit at high plasma concentrations of a drug (transport maximum), and substances with similar physicochemical properties may compete for excretion via the same mechanism.

Drugs with a mol wt > 300 g/mol (smaller molecules are generally excreted only in negligible amounts) and with both polar and lipophilic groups are more likely to be excreted in bile. Conjugation, particularly with glucuronic acid, also leads to biliary excretion.

In the **enterohepatic cycle,** a drug secreted in bile is reabsorbed from the intestine. Drug conjugates secreted into the intestine also undergo enterohepatic cycling when they are hydrolyzed and the drug is reabsorbed. Biliary excretion eliminates substances from the body only to the extent that enterohepatic cycling is incomplete, ie, when some of the secreted drug is not reabsorbed from the intestine.

299 / PHARMACOKINETICS

Study of the time course of a drug and its metabolites in the body after administration by any route.

An appropriate response to a drug requires the appropriate concentration of drug at the site of action. The dosage regimen required to attain and maintain the appropriate concentration depends on pharmacokinetics. The appropriate concentration and dosage regimen depend on the patient's clinical state, severity of the disorder, presence of concurrent disease, use of other drugs, and other factors.

Because of individual differences, drug administration must be based on each patient's needs—traditionally, by empirically adjusting dosage until the therapeutic objective is met. This approach is frequently inadequate because optimal response may be delayed or serious toxic reactions may occur. Alternatively, a drug can be administered according to its expected absorption and disposition (distribution and elimination—see also Ch. 298) in a patient, and dosage can be adjusted by monitoring plasma drug concentration and drug effects. This approach requires knowledge of the drug's pharmacokinetics as a function of the patient's age and weight and the kinetic consequences of concurrent diseases (eg, renal, hepatic, or cardiovascular disease or a combination of diseases).

BASIC PHARMACOKINETIC PARAMETERS

The pharmacokinetic behavior of most drugs can be summarized by the following parameters, whose formulas are listed in TABLE 299–1. The parameters are constants, although their values may differ from patient to patient and in the same patient under different conditions.

Bioavailability expresses the extent of drug absorption into the systemic circulation (see Ch. 298). The **absorption rate constant** expresses the speed of absorption. These parameters influence the maximum (peak) concentration, the time at which the maximum concentration occurs (peak time), and the area under the concentration-time curve (AUC) after a single oral dose. During long-term drug therapy, the extent of absorption is the more important measurement because average concentration depends on it; the degree of fluctuation is related to the absorption rate constant.

The **apparent volume of distribution** is the amount of fluid that would be required to contain the drug in the body at the same concentration as in the blood or plasma. It can be used to estimate the dose required to produce a given concentration and the concentration expected for a given dose. The unbound concentration is closely associated with drug effects, so **unbound fraction** is a useful measure, particularly when plasma protein binding is altered—eg, by hypoalbuminemia, renal or hepatic disease, or displacement interactions. The apparent volume of distribution and the unbound fraction in plasma are the most widely used parameters for drug distribution (see Ch. 298).

The rate of elimination of a drug from the body varies with the plasma concentration. The parameter relating elimination rate to plasma concentration is **total clearance,** which equals renal clearance plus extrarenal

TABLE 299–1. FORMULAS DEFINING BASIC PHARMACOKINETIC PARAMETERS

Category	Parameter	Formula
Absorption	Absorption rate contant	= Rate of drug absorption ÷ Amount of drug remaining to be absorbed
	Bioavailability	= Amount of drug absorbed ÷ Drug dose
Distribution	Apparent volume of distribution	= Amount of drug in body ÷ Plasma drug concentration
	Unbound fraction	= Plasma concentration of unbound drug ÷ Plasma drug concentration
Elimination	Rate of elimination	= Renal excretion + Extrarenal (usually metabolic) elimination
	Clearance	= Rate of drug elimination ÷ Plasma drug concentration
	Renal clearance	= Rate of renal excretion of drug ÷ Plasma drug concentration
	Metabolic clearance	= Rate of drug metabolism ÷ Plasma drug concentration
	Fraction excreted unchanged	= Rate of renal excretion of drug ÷ Rate of drug elimination
	Elimination rate constant	= Rate of drug elimination ÷ Amount of drug in body
		= Clearance ÷ Volume of distribution
	Biologic half-life	= 0.693 ÷ Elimination rate constant

(metabolic) clearance (see also Estimation of Parameter Values in Ch. 303).

The **fraction excreted unchanged** helps assess the potential effect of renal and hepatic diseases on drug elimination. A low fraction indicates that hepatic metabolism is the likely mechanism of elimination and that hepatic disease may therefore affect drug elimination. Renal diseases produce greater effects on the kinetics of drugs with a high fraction excreted unchanged.

The extraction rate of a drug from the blood by an eliminating organ, such as the liver, cannot exceed the rate of drug delivery to the organ. Thus, clearance has an upper limit, based on drug delivery and hence on blood flow to the organ. Furthermore, when the eliminating organ is the liver or gut wall and a drug is given orally, part of the dose may be metabolized as it passes through the tissues to the systemic circulation; this process is called **first-pass metabolism.** Thus, if extraction (clearance) of a drug is high in the liver or gut wall, oral bioavailability is low, sometimes precluding oral administration or requiring an oral dose much larger than an equivalent parenteral dose. Drugs with extensive first-pass metabolism include alprenolol, hydralazine, isoproterenol, lidocaine, meperidine, morphine, nifedipine, nitroglycerin, propranolol, testosterone, and verapamil.

The **elimination rate constant** is a function of how a drug is cleared from the blood by the eliminating organs and how the drug distributes throughout the body.

Half-life (elimination) is the time required for the plasma drug concentration or the amount of drug in the body to decrease by 50%. For most drugs, half-life remains the same regardless of how much drug is in the body. Exceptions include phenytoin, theophylline, and heparin.

Mean residence time (MRT), another measure of drug elimination, is the average time a drug molecule remains in the body after rapid IV injection. Like clearance, its value is independent of dose. After an IV bolus,

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

AUMC is the area under the first moment of the plasma concentration–time curve. For a drug with one-compartment distribution characteristics, MRT equals the reciprocal of the elimination rate constant:

DRUG ADMINISTRATION

The kinetic consequences of administering a drug in a single dose (IV or oral), by constant-rate infusion, and in multiple oral doses are described below, using theophylline (given as aminophylline) as an example. The metabolism of theophylline is concentration-dependent in some persons, especially children. In this example, the drug is given to a 70-kg patient (patient A) who has concentration-independent metabolism and the following parameters: bioavailability, 1.0; absorption rate constant, 1.0/h; apparent volume of distribution, 0.5 L/kg; clearance, 43 mL/h/kg; and half-life, 8 h.

Single Dose

Intravascular: After a single 320-mg IV dose of aminophylline (hydrous form is 80% theophylline) is given to patient A (see FIG. 299–1), the predicted initial plasma concentration of theophylline is 7.3 mg/L (41 μmol/L)—ie, dose (256 mg) divided by apparent volume of distribution (0.5 L/kg × 70 kg = 35 L). The subsequent decline is estimated from the half-life; every 8 h, the concentration decreases by a factor of 2.

The discrepancy between the observed (solid line) and predicted (broken line) concentration-time profiles in the first 2 h is explained by the time required to distribute the drug throughout the body (distribution phase). Because drug distribution requires time, single IV doses of many drugs, including aminophylline, must be given by short-term infusion over ≥ 5 to 10 min to avoid side effects.

Extravascular: After a single 300-mg oral dose of aminophylline (anhydrous form, often used orally, is 85% theophylline) is given to patient A (see FIG. 299–2), the time course differs from that of a single IV dose (see FIG. 299–1) because time is required to absorb the drug. However, AUC is the same because this drug is virtually completely absorbed. The more rapid the absorption, the closer the curve is to that of the IV dose. The time of peak concentration is when the absorption rate equals the elimination rate; absorption is not complete at this time.

Constant-Rate Infusion

Plateau concentration: In patient A, after an IV infusion of aminophylline at a constant rate of 45 mg/h (see curve A in FIG. 299–3), the plasma concentration and amount of theophylline in the body increase until the elimination rate equals the infusion rate. The plasma concentration and the amount of drug in the body are then at steady state—a plateau. Based on the formulas for clearance and elimination rate constant (see TABLE 299–1), infusion rate equals clearance times plateau plasma drug concentration or equals elimination rate constant times pla-

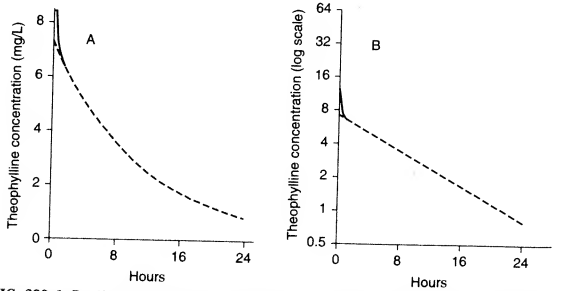

FIG. 299–1. Decline of plasma theophylline concentration in patient A after IV administration of a single 320-mg dose of aminophylline. Shown on linear (A) and semilogarithmic (B) plots. Observed curve = (——); predicted curve from given parameter values = (– – –).

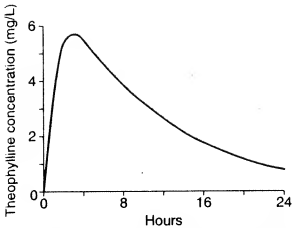

FIG. 299–2. Time course of plasma theophylline concentration after oral administration of a single 300-mg dose of aminophylline to patient A.

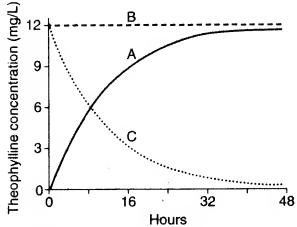

FIG. 299–3. Time course of plasma theophylline concentration after a 45-mg/h constant-rate IV infusion of aminophylline without and with a 530-mg IV loading dose in patient A. A = without loading dose; B = with loading dose; C = drug remaining from loading dose.

teau amount of drug in the body. Thus, the plateau plasma concentration is determined only by clearance and infusion rate, and the plateau amount of drug in the body is determined only by the elimination rate constant and infusion rate.

Time to reach plateau: The time required for theophylline to accumulate in the body (and then to disappear) depends on the drug's half-life, as shown in FIG. 299–3. A single IV 530-mg bolus of aminophylline produces a theophylline concentration of 12 mg/L (67 μmol/L); the bolus is followed immediately by an infusion of 45 mg/h to maintain the initial concentration (curve B in FIG. 299–3). Drug from the loading dose disappears (curve C), with 1/2 remaining at one half-life, 1/4 at two half-lives, and so on. Without the loading dose, the amount of drug in the body from the infusion (curve A) increases so that 1/2 of the plateau amount is present at one half-life, 3/4 at two half-lives, and so on.

If the infusion is stopped at 48 h, the post-infusion curve resembles curve C. Without a loading dose, aminophylline must be infused for at least 32 h (four half-lives) for the concentration to approach plateau in patient A. A plasma concentration measurement made after the plateau estimates theophylline clearance.

The principles for IV infusion apply to any constant-rate input (eg, to constant-rate devices used in transdermal, intraocular, oral, and intrauterine drug delivery). Plateau plasma concentration and the time to reach it depend on clearance and half-life, respectively, as for IV infusions. Bioavailability is an additional factor applicable to extravascular administration.

Multiple Oral Doses

Drug accumulation: Repetitive administration of aminophylline 300 mg po q 6 h to patient A increases the theophylline concentration (see curve A in FIG. 299–4). As with

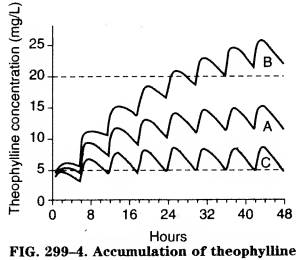

FIG. 299–4. Accumulation of theophylline after oral administration of aminophylline 300 mg q 6 h. Curve A = patient A; curve B = patient B, whose clearance is 1/2 that of patient A; curve C = patient C, whose clearance is twice that of patient A. The dashed lines are the usual therapeutic limits, representing the therapeutic window.

IV infusion, the average plateau concentration depends on clearance, and the time required for the drug to accumulate depends on half-life. Here, however, plasma concentrations fluctuate because dosing is intermittent.

If theophylline clearance is altered, eg, by disease, pharmacokinetics change (curves B and C). Patient B has heart failure with a clearance of 21.5 mL/h/kg (about half that of patient A). After patient B is given aminophylline 300 mg q 6 h, drug concentration is double that of patient A (curve B), and the time to reach plateau levels is twice as long because half-life (16 h) is twice that in a healthy adult. Plasma theophylline concentrations of 10 to 20 mg/L (55 to 110 μmol/L) are usually optimal. Above 20 mg/L, toxicity is more likely. Thus, patient B is at risk of toxicity (nausea, vomiting, CNS stimulation, seizures), which, with the knowledge that heart failure decreases metabolism, may be averted by giving a smaller dose. Also, slow metabolism may be detected by monitoring plasma concentration.

Dosage regimens: For patient B, aminophylline 200 mg q 8 h (25 mg/h) is probably appropriate. However, because of the long half-life and the slow accumulation in this patient, a loading dose must be given to rapidly produce a therapeutic concentration (and response). The required loading dose of aminophylline is the apparent volume of distribution times the desired theophylline concentration, corrected by the fraction of theophylline in aminophylline, or about 500 mg:

$$35\ \text{L} \times \frac{12\ \text{mg}}{\text{L}} \times \frac{100\ \text{mg aminophylline}}{85\ \text{mg theophylline}}$$

In a young, otherwise healthy asthmatic adult who is a heavy smoker (patient C), theophylline clearance is 86 mL/h/kg, and half-life is 4 h. Aminophylline 300 mg q 6 h (50 mg/h) is probably ineffective (see curve C in FIG. 299–4). The need for more drug can be anticipated and may be confirmed by measuring plasma concentration just before the next dose. However, giving aminophylline to this patient is difficult because of the short half-life, high clearance, and large dosage requirements (100 mg/h). For this patient, a prolonged-release formulation is indicated. Because absorption is more or less sustained, 600 mg q 6 h will probably prevent concentrations from fluctuating widely.

VARIABILITY IN PARAMETER VALUES

Many factors affecting pharmacokinetic parameters should be considered when tailoring drug administration for a particular patient. Even with dosage adjustment, however, sufficient variability usually remains; thus, drug response and, in some cases, plasma drug concentration must be closely monitored.

Age and weight: For some drugs, the effects of age and weight on pharmacokinetics are well established. For persons aged 6 mo to 20 yr, renal function appears to correlate well with BSA. Thus, for drugs primarily eliminated unchanged by renal excretion, clearance in children varies with age according to change in BSA. For persons > 20 yr, renal function decreases about 1%/yr. Thus, dosage of these drugs can be adjusted by age. BSA also correlates with metabolic clearance in children, although exceptions are common. For newborns and infants, renal and hepatic functions are not fully developed, and generalizations, except for the occurrence of rapid change, are less accurate.

Renal function impairment: Renal clearance of most drugs appears to vary directly with creatinine clearance, regardless of which renal disease is present. The change in total clearance depends on the contribution of the kidneys to total elimination. Thus, total clearance should be proportional to renal function (creatinine clearance) for drugs excreted unchanged and to be unaffected for drugs eliminated by metabolism.

Renal failure may change the apparent volume of distribution, which decreases for digoxin because of decreased tissue binding and increases for phenytoin, salicylic acid, and many other drugs because of decreased binding to plasma proteins.

Physiologic stress: Concentration of the acute-phase protein α_1-acid glycoprotein increases during physiologic stress (eg, MI, surgery, ulcerative colitis, Crohn's disease). Consequently, the binding of several drugs (eg, propranolol, quinidine, disopyramide) to this protein increases, and the apparent volume of distribution of these drugs decreases accordingly.

Hepatic disease: Hepatic dysfunction can change metabolic clearance, but good correlates or predictors of the changes are

unavailable. Hepatic cirrhosis can dramatically reduce drug metabolism and often results in reduced plasma protein binding because of lowered plasma albumin. Acute hepatitis, with elevated serum enzymes, usually does not alter drug metabolism.

Other diseases: Heart failure, pneumonia, hyperthyroidism, and many other diseases can alter the pharmacokinetics of drugs.

Drug interactions: Pharmacokinetic parameter values and, therefore, drug response may be affected by drug interactions. Most interactions are graded, and the extent of the interaction depends on the concentrations of both drugs. Thus, determining and adjusting drug dosage is difficult (see DRUG INTERACTIONS in Ch. 301).

Dosage: In some instances, changes in dose, dosing rate, or duration of therapy alter a drug's kinetics. For example, as dose is increased, the bioavailability of griseofulvin decreases because of the drug's low solubility in the fluids of the upper GI tract. For phenytoin, steady-state plasma concentration increases disproportionately when dosing rate is increased, because the metabolizing enzyme has a limited capacity to eliminate the drug, and the usual dosing rate approaches the maximum rate of metabolism. Plasma carbamazepine concentration decreases during long-term use because carbamazepine induces its own metabolism. Other causes of dosage-dependent kinetic changes are saturable plasma protein and tissue binding (eg, phenylbutazone), saturable secretion in the kidneys (eg, high-dose penicillin), and saturable metabolism during the first pass through the liver (eg, propranolol).

300 / PHARMACODYNAMICS

Study of the biochemical and physiologic effects of drugs and their mechanisms of action.

Many drugs produce pharmacologic responses by interacting with (binding to) specific macromolecules, usually complex proteins, on or within cells. Some drug classes react directly with endogenous or exogenous nonprotein substances; included are some cancer chemotherapeutic drugs that interact with nucleic acids, metal chelating drugs (eg, calcium disodium edetate, dimercaprol, deferoxamine), and antacids used to chemically neutralize gastric acid.

DRUG-RECEPTOR INTERACTIONS

Few if any drugs have absolute specificity, but most have relative selectivity; eg, atropine inhibits the actions of acetylcholine on exocrine glands and smooth muscles, but not on skeletal muscle. The action of such selective drugs results from their physicochemical binding to cellular components called receptors. Physiologic receptors are macromolecules involved in chemical signaling between and within cells. A molecule that binds to a receptor is called a ligand. When a ligand (hormone, neurotransmitter, intracellular messenger molecule, or exogenous drug) combines with a receptor, cell function changes (see TABLE 300–1). Each ligand may interact with multiple receptor subtypes. Activated receptors directly or indirectly regulate cellular biochemical processes (eg, ion conductance, protein phosphorylation, DNA transcription). In many cases, receptors within the cell membrane are coupled through guanine nucleotide-binding proteins (G proteins) to various effector systems involving intracellular second messenger molecules.

Receptors are dynamic, influenced by external factors as well as by intracellular regulatory mechanisms. Receptor up-regulation and down-regulation are relevant to clinically important adaptation to drugs (desensitization, tachyphylaxis, tolerance, acquired resistance, postwithdrawal supersensitivity).

Recognition sites are the precise molecular regions of receptor macromolecules to which ligands bind. A drug may interact at the same site as an endogenous agonist (hormone or neurotransmitter) or at a different site. Agonists that bind to an adjacent or a different site are sometimes termed allo-

US006611707B1

(12) **United States Patent**
Prausnitz et al.

(10) **Patent No.: US 6,611,707 B1**
(45) **Date of Patent: Aug. 26, 2003**

(54) **MICRONEEDLE DRUG DELIVERY DEVICE**

(75) Inventors: **Mark R. Prausnitz**, Decatur, GA (US); **Mark G. Allen**, Atlanta, GA (US); **Inder-Jeet Gujral**, Cambridge, MA (US)

(73) Assignee: **Georgia Tech Research Corporation**, Atlanta, GA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/452,979**

(22) Filed: **Dec. 2, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/137,621, filed on Jun. 4, 1999.

(51) **Int. Cl.**[7] **A61N 1/30**

(52) **U.S. Cl.** **604/21**; 604/272

(58) **Field of Search** 424/424, 449; 604/191, 186, 20, 22, 35, 46, 181, 183, 239, 261, 272, 890.1

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Primary Examiner—Manuel Mendez
(74) *Attorney, Agent, or Firm*—Ropes & Gray

(57) **ABSTRACT**

Simple microneedle devices for delivery of drugs across or into biological tissue are provided, which permit drug delivery at clinically relevant rates across or into skin or other tissue barriers, with minimal or no damage, pain, or irritation to the tissue. The devices include a substrate to which a plurality of hollow microneedles are attached or integrated, and at least one reservoir, containing the drug, selectably in communication with the microneedles, wherein the volume or amount of drug to be delivered can be selectively altered. The reservoir can be formed of a deformable, preferably elastic, material. The device typically includes a means, such as a plunger, for compressing the reservoir to drive the drug from the reservoir through the microneedles. In one embodiment, the reservoir is a syringe or pump connected to the substrate.

25 Claims, 4 Drawing Sheets

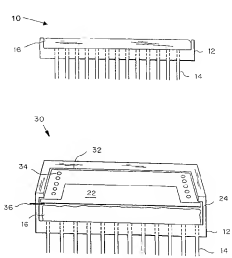

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FIG. 1A

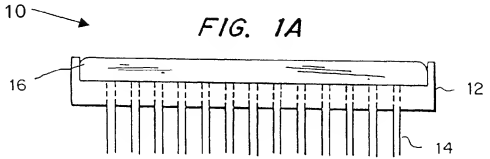


FIG. 1B

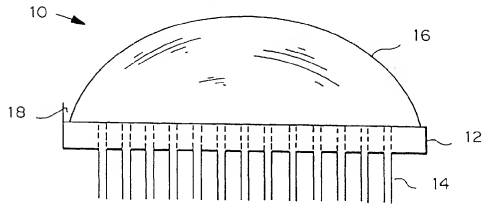
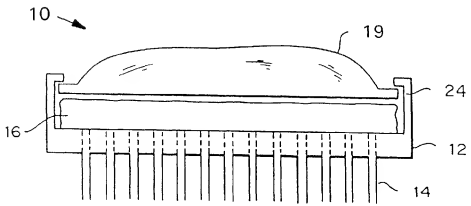
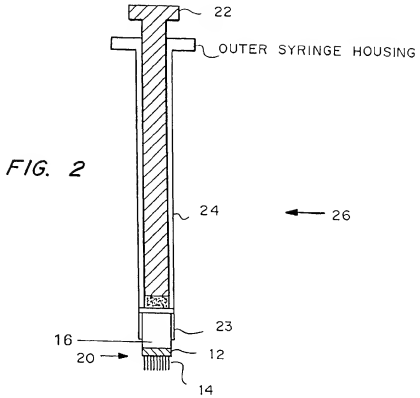


FIG. 1C

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OUTER SYRINGE HOUSING
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FIG. 2

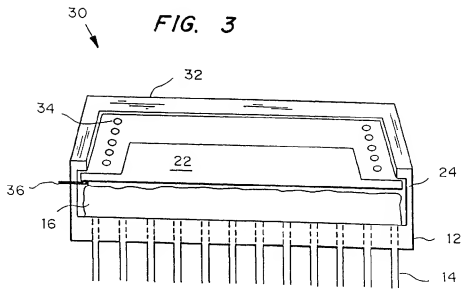
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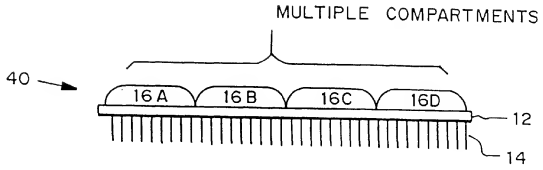
FIG. 3


MULTIPLE COMPARTMENTS
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16A
16B
16C
16D
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FIG. 4A

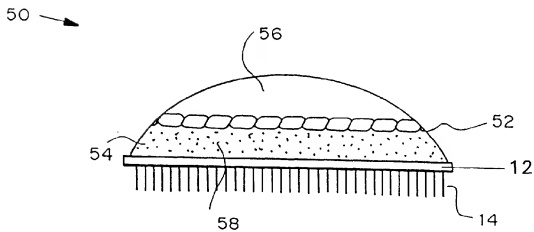
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FIG. 4B

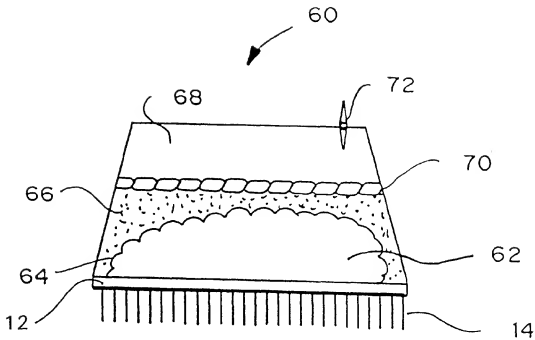

FIG. 5

MICRONEEDLE DRUG DELIVERY DEVICE

CROSS-REFERENCE TO RELATED APPLICATIONS

Priority is claimed to U.S. provisional application Serial No. 60/137,621, filed Jun. 4, 1999.

BACKGROUND OF THE INVENTION

A common technique for delivering drugs across or into biological tissue is the use of a needle, such as those used with standard syringes or catheters, to transport drugs across (through) the skin. While effective for this purpose, needles generally cause pain; local damage to the skin at the site of insertion; bleeding, which increases the risk of disease transmission; and a wound sufficiently large to be a site of infection. Needle techniques also generally require administration by one trained in its use. The needle technique also is undesirable for long term, controlled continuous drug delivery.

An alternative delivery technique is the transdermal patch, which usually relies on diffusion of the drug across the skin. However, this method is not useful for many drugs, due to the poor permeability (i.e. effective barrier properties) of the skin. The rate of diffusion depends in part on the size and hydrophilicity of the drug molecules and the concentration gradient across the stratum corneum. Few drugs have the necessary physiochemical properties to be effectively delivered through the skin by passive diffusion. Iontophoresis, electroporation, ultrasound, and heat (so-called active systems) have been used in an attempt to improve the rate of delivery. While providing varying degrees of enhancement, these techniques are not suitable for all types of drugs, failing to provide the desired level of delivery. In some cases, they are also painful and inconvenient or impractical for continuous controlled drug delivery over a period of hours or days.

Attempts have been made to design alternative devices for active transfer of drugs, but there remains a need for better drug delivery devices, which make smaller incisions, deliver drug with greater efficiency (greater drug delivery per quantity applied) and less variability of drug administration, and/or are easier to use.

It is therefore an object of the present invention to provide a microneedle device for relatively painless, controlled, safe, convenient transdermal delivery of a variety of drugs.

SUMMARY OF THE INVENTION

Simple microneedle devices are provided for delivery of drugs across or into biological tissue, particularly the skin. The microneedle devices permit drug delivery at clinically relevant rates across or into skin or other tissue barriers, with minimal or no damage, pain, or irritation to the tissue.

The devices include a plurality of hollow microneedles, which are attached to or integrated into a substrate, and at least one reservoir selectably in communication with the microneedles, wherein the volume or amount of drug to be delivered can be selectively altered. The reservoir contains the drug to be delivered. In one embodiment, the reservoir is formed of a deformable, preferably elastic, material.

The device also can include means for compressing the reservoir to drive the drug from the reservoir through the microneedles. The means can include a plunger or osmotic pump. In one embodiment, the reservoir is a syringe or pump connected to the substrate.

The device also can include a sealing mechanism to contain the drug in one or more of the reservoirs until it is ready to be delivered or mixed with a liquid carrier. In one embodiment, the sealing mechanism is a fracturable barrier interposed between the reservoir and the substrate.

In one embodiment, the device includes a means for providing feedback to the user to indicate that delivery has been initiated and/or completed. An example of the feedback means is a color change.

In another embodiment, the microneedle device further includes a rate control means, such as a semi-permeable membrane, to regulate the rate or extent of drug which flows through the microneedles.

The microneedle devices preferably are provided with means for preventing undesired reuse of or contact with the microneedles. These means can include protective packaging, such as a peelable liner that temporarily covers the tips of the microneedles. The packaging also can be used to shear off the microneedles following their intended use, thereby preventing their reuse.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1***a–c* are cross-sectional views of preferred embodiments of a microneedle drug delivery device. The devices of FIGS. **1***a–c* each include a reservoir and are suitable for transdermal drug delivery. The device of FIGS. **1***a* and **1***b* includes a deformable reservoir, wherein delivery is activated by manual, e.g., finger or thumb, pressure applied to compress the reservoir directly (**1***b*) or indirectly (**1***c*).

FIG. **2** is a cross-sectional view of another preferred embodiment of a microneedle drug delivery device, wherein delivery is activated by manual pressure applied via a plunger to compress the reservoir.

FIG. **3** is a cross-sectional view of another preferred embodiment of a microneedle drug delivery device, wherein delivery is activated by releasing a compressed spring which forces the plunger to compress the reservoir.

FIGS. **4***a–b* are cross-sectional views of preferred embodiments of a microneedle drug delivery device having a multiple chambered reservoirs.

FIG. **5** is a cross-sectional view of a preferred embodiment of the microneedle drug delivery device, which incorporates an osmotic pump to force the drug contents from the reservoir.

DETAILED DESCRIPTION OF THE INVENTION

The microneedle devices include at least three components: at least one, more preferably more, microneedle(s); a substrate to which the base of the microneedle(s) are secured or integrated; and at least one reservoir that is selectably in fluid connection with one or more of the microneedles. Preferably, the microneedles are provided as a multi-dimensional array, in contrast to a device with a single microneedle or single row of microneedles. The microneedle device can be adapted to be a single-use, disposable device, or can be adapted to be fully or partially reusable.

Microneedles

The microneedles are hollow; that is, each contains at least one substantially annular bore or channel having a diameter large enough to permit passage of a drug-containing fluid and/or solid materials through the microneedle. The hollow shafts may be linear, i.e. extend upwardly from needle base to needle tip, or they may take a more complex path, e.g. extend upwardly from the needle base,

but then lead to one or more 'portholes' or 'slits' on the sides of the needles, rather than an opening at the needle tip.

The microneedles can be constructed from a variety of materials, including metals, ceramics, semiconductors, organics, polymers, and composites. Preferred materials of construction include pharmaceutical grade stainless steel, gold, titanium, nickel, iron, tin, chromium, copper, palladium, platinum, alloys of these or other metals, silicon, silicon dioxide, and polymers. Representative biodegradable polymers include polymers of hydroxy acids such as lactic acid and glycolic acid polylactide, polyglycolide, polylactide-co-glycolide, and copolymers with PEG, polyanhydrides, poly(ortho)esters, polyurethanes, poly (butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone) Representative non-biodegradable polymers include polycarbonate, polyester, and polyacrylamides.

The microneedles should have the mechanical strength to remain intact while being inserted into the biological barrier, while remaining in place for up to a number of days, and while being removed. In embodiments where the microneedles are formed of biodegradable polymers, the microneedle must to remain intact at least long enough for the microneedle to serve its intended purpose (e.g., its conduit function for delivery of drug). The microneedles should be sterilizable using standard methods such as ethylene oxide or gamma irradiation.

The microneedles can have straight or tapered shafts. In a preferred embodiment, the diameter of the microneedle is greatest at the base end of the microneedle and tapers to a point at the end distal the base. The microneedle can also be fabricated to have a shaft that includes both a straight (untapered) portion and a tapered portion. The needles may also not have a tapered end at all, i.e. they may simply be cylinders with blunt or flat tips. A hollow microneedle that has a substantially uniform diameter, but which does not taper to a point, is referred to herein as a "microtube." As used herein, the term "microneedle" includes both microtubes and tapered needles unless otherwise indicated.

The microneedles can be oriented perpendicular or at an angle to the substrate. Preferably, the microneedles are oriented perpendicular to the substrate so that a larger density of microneedles per unit area of substrate can be provided. An array of microneedles can include a mixture of microneedle orientations, heights, or other parameters.

The microneedles can be formed with shafts that have a circular cross-section in the perpendicular, or the cross-section can be non-circular. For example, the cross-section of the microneedle can be polygonal (e.g. star-shaped, square, triangular), oblong, or another shape. The shaft can have one or more bores. The cross-sectional dimensions typically are between about 1 μm and 500 μm, and preferably between 10 and 100 μm. The outer diameter is typically between about 10 μm and about 100 μm, and the inner diameter is typically between about 3 μm and about 80 μm.

In one embodiment the cross-sectional dimensions are designed to leave a residual hole (following microneedle insertion and withdrawal) of less than about 0.2 μm, to avoid making a hole which would allow bacteria to enter the penetration wound. The actual microneedle diameter will typically be in the few micron range, since the holes typically contract following withdrawal of the microneedle. Larger diameter and longer microneedles are acceptable, so long as the microneedle can penetrate the biological barrier to the desired depth.

The length of the microneedles typically is between about 10 μm and 1 mm, preferably between 100 μm and 500 μm, and more preferably between 150 μm and 350 μm. The length is selected for the particular application, accounting for both an inserted and uninserted portion. An array of microneedles can include a mixture of microneedles having, for example, various lengths, outer diameters, inner diameters, cross-sectional shapes, and spacings between the microneedles. In transdermal applications, the "insertion depth" of the microneedles is preferably less than about 100–150 μm, so that insertion of the microneedles into the skin does not penetrate into the dermis, thereby avoiding contacting nerves which may cause pain. In such applications, the actual length of the microneedles typically is longer, since the portion of the microneedles distal the tip may not be inserted into the skin; the uninserted length depends on the particular device design and configuration. The actual (overall) height or length of microneedles should be equal to the insertion depth plus the uninserted length.

Substrate

The substrate of the device can be constructed from a variety of materials, including metals, ceramics, semiconductors, organics, polymers, and composites. The substrate includes the base to which the microneedles are attached or integrally formed. The substrate can be adapted to fit a Luer-Lock syringe or other conventionally used drug delivery device that currently uses hypodermic needles as the barrier penetration method.

In one embodiment of the device, the substrate, as well as other components, are formed from flexible materials to allow the device to fit the contours of the biological barrier, such as the skin, vessel walls, or the eye, to which the device is applied. A flexible device may facilitate more consistent penetration of some biological barriers, because penetration can be limited by deviations in the attachment surface. For example, the surface of human skin is not flat due to dermatoglyphics (i.e. tiny wrinkles) and hair. However, for some biological barriers, a rigid substrate may be preferred.

Reservoir

The reservoir is selectably in connection with the microneedle bore, such that the reservoir contents can flow from the reservoir and out through the microneedle tip, into the target tissue. Typically, it is attached to, or integrated into, the substrate, either integrally (as in a one-piece device) or at the moment of drug delivery (as with a Luer-lock type device). The reservoir is to provide suitable, leak-free storage of the drug composition before it is to be delivered. The reservoir should keep the drug composition free of contaminants and degradation-enhancing agents. For example, the reservoir should exclude light when the drug composition contains photo-sensitive materials, and should include an oxygen barrier material in order to minimize exposure of drugs sensitive to oxidation. Also, the reservoir should keep volatile materials inside the reservoir, for example, to prevent water from evaporating, causing the drug composition to dry out and become undeliverable.

The drug reservoir can be substantially rigid or readily deformable. The reservoir can be formed from one or more polymers, metals, ceramics, or combinations thereof. In a preferred embodiment, the reservoir includes a volume surrounded by one or more walls, or includes a porous material, such as a sponge, which can retain, for example, the drug liquid until the material is compressed.

In a preferred embodiment, the reservoir is formed of an elastic material, such as an elastomeric polymer or rubber. For example, the reservoir can be a balloon-like pouch that

is stretched (in tension) when filled with a fluid drug composition to be delivered.

The reservoir of a single microneedle device can include a plurality of compartments that are isolated from one another and/or from a portion of the microneedles in an array. The device can, for example, be provided to deliver different drugs through different needles, or to deliver the same or different drugs at different rates or at different times (FIG. **4***a*). Alternatively, the contents of the different compartments can be combined with one another, for example, by piercing, or otherwise removing, a barrier between the compartments, so as to allow the materials to mix. In a preferred embodiment, one compartment contains a saline solution or another delivery vehicle, while another compartment contains lyophilized drug (FIG. **4***b*). In a preferred embodiment, the reservoir is a standard or Luer-Lock syringe adapted to connect to a microneedle array.

Methods for Manufacture of the Devices

The microneedle and substrate are made by methods known to those skilled in the art. Examples include microfabrication processes, by creating small mechanical structures in silicon, metal, polymer, and other materials. Three-dimensional arrays of hollow microneedles can be fabricated, for example, using combinations of dry etching processes; micromold creation in lithographically-defined polymers and selective sidewall electroplating; or direct micromolding techniques using epoxy mold transfers. These methods are described, for example, in U.S. Ser. No. 09/095,221, filed Jun. 10, 1998; U.S. Ser. No. 09/316,229, filed May 21, 1999; Henry, et al., "Micromachined Needles for the Transdermal Delivery of Drugs," *Micro Electro Mechanical Systems*, Heidelberg, Germany, pp. 494–98 (Jan. 26–29, 1998).

Examples

Preferred embodiments of the microneedle device are shown in FIGS. **1***a–c*. The device **10** includes substrate **12** from which a three-dimensional array of microneedles **14** protrude. As shown, the annular bore of the microneedles **14** extends through the substrate **12**. The device **10** also includes a reservoir **16** secured to substrate **12** via a sealing mechanism **18**. FIG. **1***a* shows how the reservoir can be accessed directly by application to the skin, for example, for simple transdermal delivery of an agent. The device in FIG. **1***b* includes a deformable bubble reservoir **16**. Manual pressure can be used to expel its contents at the site of application. FIG. **1***c* shows a separate reservoir **16** from means **19** for expelling the contents of the reservoir **16** at the site of administration. The expelling means **19** can be simply a flexible bag. The expelling means **19** may also contain a vacuum so that it expands when vented, to create pressure on the reservoir, or it may be elastic so that it deforms when released from one position (not shown). Alternatively, reservoir **16** could be formed of an elastic material which deforms when released.

The sealing mechanism **18** can be, for example, an adhesive material or gasket. The sealing mechanism **18** can further function as or include a fracturable barrier or rate controlling membrane overlaying the surface of the substrate. In this embodiment, nothing can be released until a seal or peel-off strip covering is removed.

Another preferred embodiment of the microneedle device is shown in FIG. **2**. The device **20** includes substrate **12** from which a three-dimensional array of microneedles **14** protrude. The device **20** also includes plunger **22** that is slidably secured to the upper surface of substrate **12** by plunger guide frame **24** using a restraint such as a Luer-lock interface **23**. The substrate **12** can be attached or detached to a syringe **26** via a connector such as a Luer-lock type attachment **23**. The plunger **22**, guide frame **24**, and connector **23** connect to, form or contain reservoir **16**. A Luer-lock type attachment could alternatively be used to secure the device to means for controlling flow or transport through the device such as a pump.

A further preferred embodiment of the microneedle device is shown in FIG. **3**. Like the device in FIG. **2**, the device **30** includes substrate **12**, microneedles **14**, plunger **22**, plunger guide frame **24**, and reservoir **16**. Device **30** further includes plunger housing **32**, which is attached to, or integrally formed with, plunger guide frame **24**. A compressed spring or other tension-based mechanism **34** is positioned between plunger housing **32** and plunger **22**. The device **30** further includes spring hold/release mechanism **36**, which keeps the plunger up (spring compressed) until triggered to compress reservoir **16**.

Attachment Feature

In a preferred embodiment, the microneedle device includes an adhesive material to secure the microneedle device to the skin, temporarily immobilizing the microneedles while inserted into the skin to deliver the drug. The adhesive agent typically is applied to the substrate (in between the microneedles at their base) or to an attachment collar or tabs adjacent the microneedles.

Care must be taken so that any adhesive agent does not plug the bores of hollow microneedles. For example, the adhesive agent can be applied in a liquid solution by flooding the top of the substrate below the tips of the microneedles, such as from the side of an array of microneedles, or by using a three-dimensional printing process. The solvent can then be evaporated from the adhesive agent solution, thereby precipitating or gelling the adhesive agent to yield a tacky surface. An alternate method of keeping the tips free of an applied adhesive agent is to choose materials of construction having a hydrophobicity or hydrophilicity to control the wetting of the surface to the microneedle tips.

Initiating Delivery

In a preferred embodiment, delivery of the drug from the reservoir is initiated by applying a force, such as by pressing the top of the reservoir, to cause the reservoir contents (i.e. a drug containing composition) to flow out through the microneedles—an active or dynamic process. For example, the user can apply finger-pressure directly to a deformable reservoir "bubble," (FIG. **1**) or to a plunger mechanism (FIG. **2**) or a Luer-lock type syringe that in turn causes the drug composition to be forced from the reservoir. The plunger also can be adapted to activate by application of a constant, reproducible force, for example, a spring (e.g., under compression) (FIG. **3**) or elastic band (e.g., in tension).

A variation of this embodiment utilizes a balloon-like reservoir in tension to provide the force. Then, when an opening is formed in the balloon reservoir, the contents are forced out of the reservoir as the balloon contracts to its relaxed state. The contraction is selectively triggered to provide the driving force for delivery.

In a preferred embodiment, the force ruptures a fracturable barrier between the reservoir contents and the inlet of the microneedle. Representative barriers include thin foil,

polymer, or laminant films. In another preferred embodiment, the microneedles tips are blocked until immediately before use. The blocking material can be, for example, a peelable adhesive or gel film, which will not clog the openings in the microneedle tip when the film is removed from the device

Delivery also can be initiated by opening a mechanical gate or valve interposed between the reservoir outlet and the microneedle inlet. For example, a thin film or plate can be slid or peeled away from the back of the substrate.

In an alternate embodiment, delivery is initiated by changing the physical or chemical properties of the drug composition and/or of a barrier material. For example, the barrier can be a porous membrane having a porosity that can be selectably altered to permit flow, or the drug composition can be selected to change from a solid or semi-solid state to a fluid state, for example as the temperature is increased from ambient to that of body temperature. Such a drug composition can be prepared, for example, by combining the drug with a biodegradable polymeric material.

A preferred embodiment of the microneedle device is shown in FIG. **4***a*. FIG. **4***a* shows a device **40** in which microneedles **14** attached to a substrate **12** which is attached to multiple compartments **16***a*, **16***b*, **16***c*, and **16***d*. Each compartment can contain or function as a reservoir. Material can be expelled from each compartment through all or a subset of microneedles **14**.

FIG. **4***b* depicts a device **50** in which microneedles **14** are attached to a substrate **12** which is attached to reservoir **58** containing, for example, lyophilized drug **54**. The reservoir **58** is attached to a fracturable barrier **52** which is attached to another reservoir **56** containing, for example, saline. If the barrier **52** is fractured, then the two reservoirs **54** and **56** are in fluid communication with each other and their contents can mix.

Delivery also can be initiated by activating an osmotic pump, as described, for example, in U.S. Pat. No. 4,320,758 to Eckenhoff, which has been adapted to the substrate of the microneedle device. For example, the reservoir/osmotic pump includes an inner flexible bag that holds the drug charge, an intermediate layer of an osmotically effective solute composition, such as an inorganic salt, that encapsulates the bag, and an outer shape-retaining membrane that is at least in part permeable to water and that encapsulates both the layer of osmotically effective solute composition and the bag. In operation, the bag filled with the fluid drug compositions is exposed to an aqueous environment, so that water is imbibed from the environment by the osmotically effective solute through the membrane into the space between the inner flexible bag and the membrane. Since the bag is flexible and the membrane is rigid, the imbibed water squeezes the bag inwardly, thereby displacing drug out the microneedles.

FIG. **5** shows a device **60** in which microneedles **14** are attached to a substrate **12** which is attached to a drug reservoir **62**. This reservoir is surrounded at least partially by a flexible, impermeable membrane **64**. The drug reservoir is connected to another reservoir **66** which contains, for example, an inorganic salt. The two reservoirs **62** and **66** are separated by the impermeable membrane **64**, which is impermeable to the contents of both reservoirs **62** and **66**. The reservoir **66** is also connected to another reservoir **68** which contains, for example, an aqueous solution in which the organic salt is at least partially soluble. The two reservoirs **66** and **68** are separated by a rigid, semi-permeable membrane **70**, which is partially or completely impermeable to the salt in reservoir **66** and partially or fully permeable to the solution in reservoir **68**. There is also an optional fill port or vent **72** in communication with the reservoir **68**, through which material can be added to or removed from the reservoir **68**. Using this device **60**, water can be drawn from the reservoir **68** across the semi-permeable membrane **70** into the reservoir **66** due to osmosis caused by the presence of salt in the reservoir **66**. The flow of water will cause the volume of reservoir **66** to increase and thereby force the volume of reservoir **62** to decrease, which causes material to expel from reservoir **62** through microneedles **14**.

In an alternate embodiment, delivery is initiated by opening the pathway between the reservoir and the microneedle tip, or unblocking the tip openings, and simply allowing the drug to be delivered by diffusion, that is, a passive process.

Feedback About Delivery

In a preferred embodiment, the microneedle device includes a feedback means so that the user can (1) determine whether delivery has been initiated; and/or (2) confirm that the reservoir has been emptied, that is delivery complete. Representative feedback means include a sound, a color (change) indicator, or a change in the shape of a deformable reservoir. In a preferred embodiment, the feedback for completion of delivery is simply that the reservoir is pressed flat against the back of the substrate and cannot be further deformed.

Feedback About Penetration of the Microneedles Into the Tissue

The user of the microneedle device typically can determine if the microneedles have been properly inserted into the skin or other tissue through visual or tactile means, that is assessing whether the substrate has been pressed essentially flush to the tissue surface. For example, if a puddle of liquid drug composition appears near the device, then the user may infer that the microneedles are not fully inserted, suggesting that the device needs to be reapplied. The liquid drug compositions can include a coloring agent to enhance the visual feedback.

In a more complex embodiment, an electrical or chemical measurement is adapted to provide the feedback. For example, penetration can be determined by measuring a change in electrical resistance at the skin or other tissue, or a pH change. Alternately, needle-to-needle electrical resistance can be measured. In a preferred embodiment, the microneedle device includes a disposable cartridge containing the microneedles. In these devices, an LED (e.g. green light/red light) or liquid crystal display can be provided with the reusable portion of the device.

Controlling the Delivery Rate

The microneedle device must be capable of transporting drug across or into the tissue at a useful rate. For example, the microneedle device must be capable of delivering drug at a rate sufficient to be therapeutically useful. The rate of delivery of the drug composition can be controlled by altering one or more of several design variables. For example, the amount of material flowing through the needles can be controlled by manipulating the effective hydrodynamic conductivity (the volumetric through-capacity) of a single device array, for example, by using more or fewer microneedles, by increasing or decreasing the number or diameter of the bores in the microneedles, or by filling at least some of the microneedle bores with a diffusion-limiting material. It is preferred, however, to simplify the manufac-

turing process by limiting the needle design to two or three "sizes" of microneedle arrays to accommodate, for example small, medium, and large volumetric flows, for which the delivery rate is controlled by other means.

Other means for controlling the rate of delivery include varying the driving force applied to the drug composition in the reservoir. For example, in passive diffusion systems, the concentration of drug in the reservoir can be increased to increase the rate of mass transfer. In active systems, for example, the pressure applied to the reservoir can be varied, such as by varying the spring constant or number of springs or elastic bands.

In either active or passive systems, the barrier material can be selected to provide a particular rate of diffusion for the drug molecules being delivered through the barrier at the needle inlet.

Drugs

Essentially any drug can be delivered using the microneedle devices described herein. As used herein, the term "drug" refers to an agent which possesses therapeutic, prophylactic, or diagnostic properties in vivo, for example when administered to an animal, including mammals, such as humans. Examples of suitable therapeutic and/or prophylactic active agents include proteins, such as hormones, antigens, and growth factors; nucleic acids, such as antisense molecules; and smaller molecules, such as antibiotics, steroids, decongestants, neuroactive agents, anesthetics, and sedatives. Examples of suitable diagnostic agents include radioactive isotopes and radioopaque agents, metals, gases, labels including chromatographic, fluorescent or enzymatic labels.

The drug can be or include a peptide, protein, carbohydrate (including monosaccharides, oligosaccharides, and polysaccharides), nucleoprotein, mucoprotein, lipoprotein, glycoprotein, nucleic acid molecules (including any form of DNA such as cDNA, RNA, or a fragment thereof, oligonucleotides, and genes), nucleotide, nucleoside, lipid, biologically active organic or inorganic molecules, or combination thereof.

The amount of drug can be selected by one of skill in the art, based, for example on the particular drug, the desired effect of the drug at the planned release levels, and the time span over which the drug should be released.

Multi-Cartridge Microneedle Device

A modification of the disposable, single use microneedle device utilizes a reusable triggering device (e.g., a plunger) in combination with a cartridge containing one or more, preferably a plurality, of single-use microneedle devices. For example, the cartridge can be a circular disk having 10 or 12 microneedle arrays connected to a single-dose reservoir, wherein the cartridge can be loaded into and unloaded from the triggering device. The triggering device can, for example, be designed to move a new dose into position for delivery, compress the reservoir to deliver the drug, and then eject or immobilize the used array. This type of reusable triggering device also can include a power source, such as a battery, used to operate a built-in measurement device, for example, for analyte measurement of interstitial fluids or electrical verification of needle penetration into skin, as described earlier in this document.

Microneedle Device Packaging

In a preferred embodiment following manufacture of the microneedle device, it is packaged for storage, shipment, and sale before use. The packaging should prevent contamination and damage. The packaging also should prevent premature triggering or release of any drug or vehicle contents from the reservoir.

It is particularly important that, the microneedle device is provided with a removable protective cover or cushion that protects the microneedles from damage. The protective cover also can function to keep the drug material from prematurely leaking out of the microneedles. In a preferred embodiment, an adhesive material or gel film used to selectively secure the cover over the microneedles. In an alternate embodiment, the film is antiseptic, and following removal can serve as a wipe to prepare the skin surface before insertion of the microneedles.

The packaging also can be adapted to serve as a vessel for safely disposing of the used microneedle device. In a preferred embodiment, the single-use microneedles are provided a device or material to shear the needles from the substrate or plug the microneedles, so as to prevent undesirable reuse of the device. In one embodiment, the inner back of the reservoir is provided with a tacky substance. When the reservoir is compressed against the back of the substrate following delivery of the reservoir contents, the tacky substance is driven into the opening of the microneedles, plugging them. In one embodiment of this device, the tacky material dries and hardens so that the substance cannot readily be removed.

Publications cited herein and the material for which they are cited are specifically incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Modifications and variations of the methods and devices described herein will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

We claim:

1. A device for transport of a material across or into a biological barrier comprising:

a plurality of hollow microneedles each having a base end and a tip, with at least one hollow pathway disposed at or between the base end and the tip, wherein the microneedles comprise a metal,

a substrate to which the base ends of the microneedles are attached or integrated, and

at least one reservoir which is in connection with the base ends of at least one of the microneedles, either integrally or separably until the moment of use,

wherein the volume or amount of material to be transported can selectively be altered.

2. The device of claim **1** wherein the reservoir is formed of a material which is deformable.

3. The device of claim **2** wherein the reservoir is elastic.

4. The device of claim **1** wherein the reservoir further comprises a therapeutic, prophylactic, or diagnostic agent.

5. The device of claim **4** wherein the agent is selected from the group consisting of peptides, proteins, carbohydrates, nucleic acid molecules, lipids, organic molecules, biologically active inorganic molecules, and combinations thereof.

6. The device of claim **1** wherein the diameter of the microneedles is between about 1 μm and about 100 μm.

7. The device of claim **1** wherein the length of the microneedles is between about 100 μm and 1 mm.

8. The device of claim **1** wherein the microneedles are adapted to provide an insertion depth of less than about 100 to 150 μm.

9. The device of claim 1 comprising a three dimensional array of microneedles.

10. The device of claim 1 further comprising an adhesive material for securing the device during delivery.

11. The device of claim 1 wherein at least the microneedles are manufactured for single use as a disposable reagent.

12. The device of claim 1 wherein the device further comprises means for stopping delivery.

13. The device of claim 1 wherein the substrate is formed of a flexible material.

14. The device of claim 1 in a packaging that protects the device from damage and/or contamination.

15. The device of claim 1 further comprising a removable liner that covers the microneedle tips and that can be used to protect and/or seal access to or from the microneedles.

16. The device of claim 1 wherein the device comprises rate control means, which can be used to regulate the rate or extent at which materials flow through the microneedles.

17. The device of claim 16 wherein the rate control means is a membrane at one end of the microneedles through which agent or fluid must pass.

18. The device of claim 16 wherein the rate control means is a material positioned within the hollow pathway of the microneedles.

19. The device of claim 1 comprising multiple microneedle arrays in combination with means for accessing one or more arrays at a time.

20. The device of claim 1 comprising means for preventing undesired contact with or use of the microneedles.

21. The device of claim 20 wherein the means is packaging that covers the microneedles.

22. The device of claim 20 wherein the means is packaging that shears off the microneedles, or tips thereof, after use.

23. The device of claim 20 wherein the means are materials which are delivered into the hollow pathways to seal the microneedles.

24. A kit of parts for delivering a therapeutic, prophylactic or diagnostic agent across or into tissue comprising:

(a) one or more microneedle devices which comprise a plurality of hollow microneedles comprising a metal, a substrate to the microneedles are attached or integrated, and at least one reservoir which is selectably in communication with the microneedles, wherein at least one of the reservoirs contains the therapeutic, prophylactic or diagnostic agent to be delivered; and

(b) a triggering device which is adapted to activate delivery of the agent from reservoir through the microneedles of one of the devices at a time.

25. The device of claim 6, wherein microneedles have an outer diameter between 10 and 100 microns.

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An investigation of the intradermal route as an effective means of immunization for microparticulate vaccine delivery systems

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Abstract

Among the common routes of parenteral immunization, the skin is the only site that can function as an immune organ. Skin-associated lymphoid tissue contains specialized cells that enhance the immune response. The intercellular space in the skin interstitium provides a connection to the lymphatic capillaries and vessels that terminate in peripheral immune organs like the lymph nodes and spleen. The potential of intradermal immunization with microparticulate vaccine delivery systems was investigated in this study. The microparticulates used were muramyl dipeptide (MDP) loaded ovalbumin microspheres (OVA–MSs) and fluorescent latex microspheres of fixed sizes of 2.3 and 2.1 μm diameter, respectively. Similar doses of OVA–MSs were injected subcutaneously (sc) and intradermally (id) in mice. The induced OVA-specific IgG antibody immune response was found to be significantly higher in id immunized mice as compared to those injected sc. The sc and id administration of fluorescent latex microspheres in mice demonstrated that the uptake and translocation of microspheres from the site of injection depends primarily upon the surface area of the microspheres. The enhancement in antibody production upon id administration was explained on the basis of (i) an increased surface area of microspheres and a lower number of microspheres per injection site, and (ii) an increased probability of interaction with the immune cells of the skin. Efficient lymph node targeting observed from the id administered microspheres may be the result of both of these factors. The results of this study demonstrated that the intradermal route is an effective means of immunization for microparticulate vaccine delivery systems, requiring lower doses and resulting in a higher immune response as compared to the traditionally used sc route.

Keywords: Intradermal; Subcutaneous; Immunization; Microspheres; Lymph node targeting

1. Introduction

The immunization route, adjuvant and dosage form used to vaccinate patients are known to influence patterns of epitope recognition as well as profiles of cellular and antibody responses [1–7]. Of the various injection routes, intramuscular (im) has been, by far, the most studied to date [8–12] because of the potential of this site to offer a depot effect. Muscle is not considered to be a site for antigen (Ag) presentation because it contains few, if any, dendritic cells, macrophages, and lymphocytes [13]. The subcutaneous (sc) route has the advantage of acting as a depot site for cytokines, thus allowing a longer apparent half-life in the vicinity of antigen deposition [12,14,15]. However, skin is the only parenteral site that can itself act as an immune organ [11], and, therefore, holds immense potential as an immunization route.

The first clinical trial of intradermal (id) immunization was reported in 1930 when Tuft [16–18] demonstrated that a small dose of typhoid vaccine given id was as immunogenic and produced fewer systemic reactions than a larger dose given sc. Following this

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study, numerous clinical trials investigated the optimal route of immunization [19–26]. Intradermal immunization is attractive since the target organ is easily accessible, and the immune system is well represented in both the epidermis and dermis [11]. The skin-associated lymphoid tissue contain specialized cells that enhance immune responses [13]. The keratinocytes produce interleukin-1 (IL-1) and tumor necrosis factor α, which can activate lymphocytes, macrophages, and dendritic cells (DC) [27]. The id route has the advantage of delivering a DC growth factor directly to the skin compartment where DC reside. Once DC are exposed to Ag and activated, they migrate to regional lymph nodes to activate T cells [28]. Despite offering the potential of a very effective route of immunization, id delivery of vaccines has been limited because of the practical difficulties of administration [29,30].

Apart from the administration route, the vaccine dosage form also plays a critical role in developing effective vaccination protocols. Conventional vaccines rely on multiple-dose administration of an antigen in order to induce adequate and long lasting immunity [31]. Alternative vaccine delivery systems, like microspheres (MSs), nanoparticles and liposomes have also been investigated and long lasting immunity appears to be a result of the sustained delivery of vaccine components [32]. The use of MSs to deliver vaccine immunogens represents a nontraditional adjuvant approach [33]. Protein or glycoprotein immunogens as well as adjuvants encapsulated into polymer MS formulations are delivered and released to the immune system more slowly than are soluble immunogens and adjuvants [34,35]. This approach offers the advantage of enhanced immunogenicity with a lower dose of immunogen and adjuvant. This is due to a sustained release of immunogen and adjuvant, protection of undelivered antigen (Ag) from rapid degradation in vivo, a decreased probability of unwanted side effects and reduced cost. The MSs mediated vaccine delivery also offers the advantages of a localized or targeted delivery of antigen to antigen presenting cells (APC) or the lymphatic system, encapsulation of more than one antigen, and improved patient compliance [36].

Efforts have been made to optimize and maximize MSs delivery to immunological cells and organs. These include modification of the physicochemical properties of the MSs, such as size, number of particles injected, their composition, charge and hydrophobicity [12,14,15,37–40]. Despite the obvious need, there is a dearth of reports in the literature exploring the potential of the immunization route using the microspheres.

The objective of the present study was to investigate and characterize the id route of immunization using MSs. The id route was compared with the traditionally used sc route, in terms of the induction of IgG Ab immune response. Mice were immunized with muramyl dipeptide (MDP) loaded ovalbumin microspheres (OVA–MSs) by sc and id routes. Subcutaneous injections were administered using a 26-gauge needle. Multiple id injections were administered using a novel intradermal delivery device (Drug Delivery Devices, Piscataway, NJ). MDP was used as the model adjuvant and OVA was used as the model Ag as well as the model polymer in this study. The difference in the degree of induced OVA specific IgG Ab immune response after sc and id administration was explained by a difference in the translocation efficiency of fluorescent latex MSs from the injection site (sc or id) through the interstitial tissue to the lymph nodes.

2. Materials and methods

2.1. Materials

Ovalbumin (albumin, chicken egg; Grade V), MDP (*N*-acetylmuramyl-L-alanyl-D-isoglutamine/muramyl dipeptide/adjuvant peptide), glutaraldehyde (25% aqueous solution; Grade I), olive oil (highly refined; low acidity), mouse IgG, anti-mouse IgG (whole molecule; conjugated to horseradish peroxidase), and *O*-phenylenediamine dihydrochloride (OPD) were purchased from Sigma Chemicals (St Louis, MO). FluoSpheres® carboxylate-modified latex microspheres (2.1 μm (±3.4%) in diameter) were purchased from Molecular Probes, Inc (Eugene, OR). Enzyme immunoassay/radioimmunoassay (EIA/RIA) 96 well plates were purchased from Corning Costar Corp. (Cambridge, MA). All the other reagents were of analytical grade and used as received.

2.2. Animals

Female CD-1 mice, 5–7 weeks of age, were obtained from Charles River Laboratories (Wilmington, MA). Mice were housed in groups of four and had free access to food and water throughout the study. All mouse studies were performed under approved protocols from the Use and Care of Animal Committee at Rutgers University in AAALAC accrediated facilities.

2.3. Ovalbumin microspheres (OVA–MSs) preparation

Ovalbumin microspheres (OVA–MSs) were prepared using a water/oil (w/o) emulsion technique of Tomlinson and Burger [41] with modifications. The detailed procedure is described elsewhere [42,43]. Briefly, 0.4 ml of an 8% (w/v) solution of OVA (in 10 mM phosphate buffered saline; PBS, pH 7.4) was emulsified in 125 ml of olive oil contained in a 200 ml baffled beaker, using a high speed homogenizer (Power Gen 700D, Fisher Scientific) for 15 min at 4200 rpm. This was followed

by the addition of 0.1 ml of 5% (v/v) glutaraldehyde solution to the emulsion, with continuous stirring. The aqueous droplets of OVA were hardened to form MSs by continuous stirring for 30 min more. After stirring, the suspension of OVA–MSs in olive oil was immediately transferred to a 300 ml elongated beaker, followed by the addition of 60 ml isopropanol. The suspension was stirred for 10 min at 4200 rpm. The stabilized OVA–MSs were collected by centrifugation (Beckman J2-MI Centrifuge) at 6000 *g* for 20 min. The MSs were washed four times with 30 ml isopropanol to remove adherent oil. Between each washing, the MSs were centrifuged at 6000 *g* for 20 min, the supernatant discarded and the MSs resuspended. The MSs were subsequently washed once with 30 ml water to remove the isopropanol. The washed MSs were centrifuged, resuspended in 10 ml water, freeze dried, and stored at 4°C in a dessiccator.

2.4. OVA–MSs particle size and morphology determination

The particle size and morphology of OVA–MSs was determined using scanning electron microscopy (SEM). Lyophilized OVA–MSs were fixed on aluminum stubs with conductive tape, and coated with gold-palladium for 2 min at 30 mA and 5×10^{-2} mbar in an argon atmosphere, using a BALZERS SCD 004 Sputter Coater. The MS coated stubs were then examined using SEM (S-450, Hitachi, Ltd., Tokyo, Japan). The images of MSs were downloaded to a computer and were analyzed for the MS particle size using the Image Pro Plus image analysis software (Media Cybernetics, Silver Spring, MD). An average of 50–100 microspheres were counted for each sample.

2.5. MDP loading and determination

MDP loaded OVA–MSs were prepared by dissolving MDP in an aqueous solution of OVA prior to its emulsification in oil. The amount of MDP added was kept constant at 10% (w/w) of OVA for all the experiments. The efficiency of MDP loading in the MSs was determined by an approach that utilized the preparation of non-stabilized OVA–MSs [41] and a mass-balance method [43], as described below.

The addition of glutaraldehyde does not affect MDP loading in the MSs [41]. Therefore the extent of MDP lost from OVA–MSs during the emulsification and isopropanol washings was determined by dissolving a fixed amount of non-stabilized OVA–MSs in water. Non-stabilized OVA–MSs were prepared in a similar manner as the glutaraldehyde stabilized OVA–MSs, with the only difference that the MSs were not stabilized by glutaraldehyde. To prepare non-stabilized OVA–MSs, 0.4 ml of an aqueous solution of OVA + MDP was emulsified in 125 ml olive oil, contained in a 200 ml baffled beaker, using homogenization for 60 min at 4200 rpm. After 60 min of stirring, the emulsion was transferred to a 300 ml elongated beaker. This was followed by the addition of 60 ml isopropanol to the emulsion. Stirring was continued for 15 min. The non-stabilized OVA–MSs were collected by centrifugation at 6000 *g* for 20 min. The MSs were washed four times with 30 ml isopropanol to remove adherent oil. Between each washing, the MSs were centrifuged, using the same centrifugation cycle as described above, the supernatant discarded and the microspheres resuspended. The fourth washing was followed by filtration of the MS suspension through a 0.2 μm filter. The MSs collected on the filter paper were dried overnight in a dessiccator under vacuum. A fixed amount of the non-stabilized OVA–MSs were dissolved in water, and the solution was assayed for MDP.

The amount of MDP removed from the stabilized OVA–MSs during water washing was measured from the MDP detected in the decanted water. The loading of MDP in the MSs was subsequently calculated using the mass-balance approach below. The amount of MDP lost during emulsification and isopropanol washings (using non-stabilized OVA-MSs) and the MDP lost during water washing was subtracted from the amount of MDP initially added to the OVA solution. The loading efficiency of MDP was calculated using Eq. (1).

$$\text{Loading efficiency} = \frac{\text{Actual load}(\%)}{\text{Theoretical load}(\%)} \times 100 \qquad (1)$$

2.6. HPLC assay for MDP

A reverse phase high-pressure liquid chromatography (RP–HPLC) assay was developed to quantitatively detect MDP. The method provided a linear response throughout the range of 0.025–14 μg MDP. The limit of detection was determined in terms of a signal:noise ratio of at least 2:1. The interday and intraday variability of the assay was very low, indicated by a coefficient of variation of less than 1%. The method utilized a Supelco C-18 column (25 × 4.6 cm, 5 μm). The mobile phase consisted of 2% methanol and 98% ammonium phosphate buffer (25 mM) adjusted to pH 7 with H_3PO_4. The flow rate of the mobile phase was kept constant at 1 ml/min and the absorbance was measured at 200 nm.

2.7. Mice immunization and blood collection

Mice were immunized sc and id, with MDP loaded and unloaded OVA–MSs along with positive and

negative controls to evaluate the IgG Ab immune response. The positive treatment controls were OVA and OVA+MDP injected in solution form. The negative control was PBS solution. The immunization studies were performed under an approved animal use protocol. The dose of OVA:MDP for immunizing each mouse was kept constant at 670:30 μg respectively, contained in 100 μl of PBS. The MSs were suspended in PBS with the aid of sonication for 4 min and vortexing, before injection.

The sc immunization of mice involved a single (bolus) injection, on the dorsal side of abdomen through a 26-gauge needle (Fig. 1A). Multiple (90–100) id injections were administered on the dorsal body side of anesthetized mice, using an intradermal delivery device (Drug Delivery Devices, Inc., a division of Dr. H. Yacowitz & Co, Piscataway, NJ 08854; US Patents 4771660, 5054339, 5401242). This device was equipped with a 26-gauge needle that penetrated to a depth of 1–2 mm in the mouse skin. The needle vibrates at a rate of 3000–3500 vibrations per min to facilitate the distribution of MSs in the epidermal, dermal, and to a lesser extent, in the subcutaneous tissue (Fig. 2 left). The vibration of the needle and the tube conveying the MSs suspension to the needle ensures that the MSs remain suspended in PBS. The suspension flow rate was kept constant at 500 μl/min for all the injections. The id injections were administered over the entire skin area on the dorsal body side of mice.

The mice were administered a booster with the same doses 1 month after the primary immunization. Blood was collected from the tail vein of mice at fixed periods of time (2 or 3 week intervals). Blood was allowed to clot and the harvested serum was stored at −20°C until assay. An enzyme linked immunosorbent assay (ELISA) was performed to quantitate the production of OVA specific IgG Ab.

2.8. ELISA

An ELISA assay was developed to quantitatively detect the OVA-specific IgG Ab in the sera of immunized mice. One hundred microliter of OVA solution (5 μg/ml) in carbonate/bicarbonate buffer (0.1 M, pH 9.6) was introduced into the individual wells of 96-well flat-bottom polystyrene plates. Following overnight incubation at 4°C, the plates were washed three times with PBS (pH 7.4) and coated with 200 μl of blocking solution in each well. The blocking solution consisted of 0.5% (w/v) bovine serum albumin (BSA) and 0.05% (w/v) Tween-20 in PBS (BSA/T20/PBS). The plates were incubated at 37°C for 2 h to facilitate

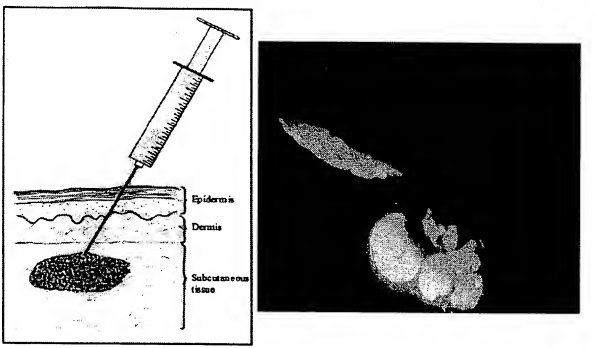

Fig. 1. (A) A schematic representation of sc administration of microspheres; (B) an aggregate of sc administered fluorescent latex microspheres in the sc tissue of mouse.

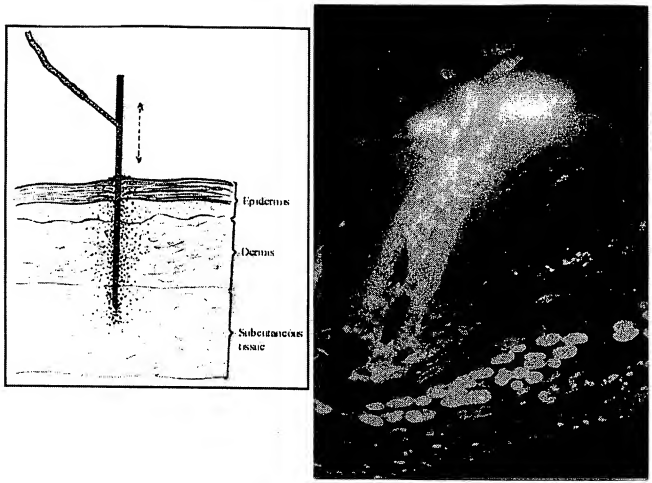

Fig. 2. (Left) A schematic representation of the distribution of id administered microspheres in various layers of mouse skin, (Right) skin section of mouse showing the distribution of the id administered fluorescent latex microspheres.

blocking, and then washed three times with PBS. Sera of immunized mice was diluted 200× in BSA/T20/PBS. Two hundred microliter of the diluted sera was introduced to the first well of each plate. Each subsequent well was coated with 100 μl of sera diluted serially in seven two-fold dilution steps. The plates were then incubated for 90 min at 37°C and washed three times with PBS. One hundred microliter of horseradish peroxidase conjugated anti-mouse IgG, diluted 2000× with BSA/T20/PBS, was added to each well of the 96 well plates. The plates were incubated for 2 h at 37°C and washed three times with PBS. Each well of the plates was then coated with 100 μl of freshly prepared *o*-phenylenediamine dihydrochloride (OPD) solution in phosphate–citrate buffer (0.05 M, pH 5.0). The color was allowed to develop for 20 min under subdued light. The reaction was stopped by the addition of 50 μl of 2N sulfuric acid in each well. The absorbance of the plates was then measured at 490 nm. The IgG response was expressed as the mean absorbance value in the linear dilution range of 1:12800 to 1:51200. All the absorbance values were normalized with respect to the negative control.

2.8.1. Statistical analysis

The results of the ELISA are expressed as mean ± standard error of mean (SEM). Differences in Ab levels were analyzed using the Student *t*-test for unpaired data. For all the pairwise comparisons, one way ANOVA was performed, followed by Student–Newman–Keuls (SNK) post hoc testing. All statistical tests were performed with a 95% confidence level.

2.9. Biodistribution of fluorescent latex MSs

Fluospheres® carboxylate-modified latex MSs (fluorescent latex MSs) were injected in mice by sc (single and multiple) and id routes. The id and single sc injections were administered as described previously for OVA–MSs. For multiple sc administration, ten injec-

tions of 10 μl each were administered on the dorsal body side of the anesthetized mouse using a Hamilton gas-tight microliter syringe (Hamilton Co, Reno, NV), equipped with a 26-gauge needle.

Following the administration of fluorescent latex MSs, three mice were euthanized from each of the three treatment groups at 1-week intervals for 5 weeks. A superficial incision was made on the ventral side of the mouse body, along the midline. Connective fascia was cut, exposing the subcutaneous tissue. Skin sections (showing visible signs of fluorescent microspheres), spleen and lymph nodes (inguinal, brachial and poplitial) were separated, fixed in 10% buffered formalin, cut into 5 μm thick sections using a microtome knife, and mounted on glass slides for fluorescent microscopy studies using a Zeiss Fluorescent Microscope.

3. Results

3.1. OVA–MSs characteristics

The OVA–MSs prepared were spherical in shape, and possessed a smooth surface, without any visible pores, as seen under the SEM. The MSs exhibited a broad size distribution, with an average diameter of 2.304 (±1.815) μm. The MDP loading efficiency in the OVA–MSs was 48.2 (±16.78) %.

3.2. Route of immunization

Fig. 1A shows a schematic representation of the accumulation of sc administered MSs in a mouse. Fig. 1B shows the aggregate of the sc administered fluorescent latex MSs in the mouse. Fig. 2 (left) schematically represents the distribution of the id administered MSs in the epidermis, dermis and the sc tissue of a mouse. The vibration of the injecting needle in the vertical motion facilitates this distribution of MSs. Fig. 2 (right) shows the distribution of the id administered fluorescent latex MSs in the various skin layers of the mouse. These figures demonstrate that the surface area of the id administered MSs exposed to adjacent tissue is much greater as compared to the sc administered MSs.

3.3. Mice immunization with OVA–MSs

The OVA specific IgG Ab immune response induced in mice by the MDP loaded and unloaded OVA–MSs was compared to the conventional solution form of the Ag, OVA and the adjuvant, MDP, as displayed in Fig. 3. The figure shows the Ab response induced 3

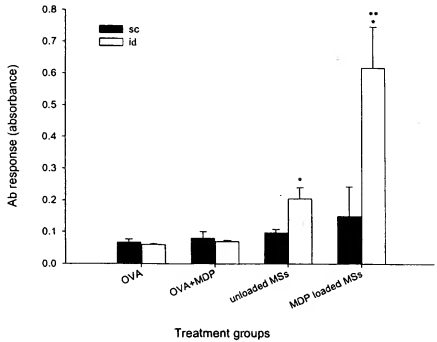

Fig. 3. OVA-specific IgG Ab immune response induced in mice 3 weeks after sc and id immunization. Positive control groups injected with OVA and OVA + MDP solution in PBS. Treatment groups injected with MDP loaded and unloaded OVA–MSs. Error bars represent ±SEM ($n = 4$ mice for sc and 10 mice for id immunization). * Ab response significantly ($p < 0.05$) different between sc and id administration of MSs; ** Ab response significantly ($p < 0.01$) different between the id administered MDP loaded and unloaded MSs.

weeks after the sc and id immunization. The administration of OVA and MDP in the MS form enhanced the production of anti-OVA IgG Ab compared to that in the solution form, irrespective of the route of immunization. The increase in the Ab production was significantly higher ($p < 0.05$) after the id administration of MSs, as compared to the sc administration. Also, the Ab response induced by the id administered MDP loaded OVA–MSs was significantly higher ($p < 0.01$) as compared to the unloaded MSs, administered id. However, the Ab response induced by the solution form of OVA and OVA + MDP was not different when injected by either sc or id routes. Fig. 4 shows the Ab immune response induced in mice, at time points of 1, 3, 5, and 9 weeks following immunization with MDP loaded OVA–MSs by the sc and id routes. The id immunization led to the induction of a significantly higher ($p < 0.05$) Ab immune response as compared to the sc immunization, at all time points. Fig. 5 shows the dose–response relationship of the MDP loaded OVA–MSs when injected by id route.

3.4. Biodistribution of fluorescent latex MSs

3.4.1. Skin distribution

Fig. 6 shows the distribution pattern of the fluorescent MSs in the skin layers of mice at time points of 7, 14 and 21 days after the id administration. The MSs were distributed throughout the various skin layers and disappeared from the site of injection after approximately 1 month. On the other hand, the MSs administered by the multiple sc injections remained aggregated near the site of injection for a time period of more than 35 days (Fig. 7). The single sc administration of MSs resulted in an aggregate formation of MSs that remained at the site of injection for a time period of more than 35 days (data not shown).

3.4.2. Lymph node targeting

The fluorescent latex microspheres targeted the inguinal, brachial and poplitial lymph nodes at various rates and extents after sc (single and multiple) and id administration. Table 1 summarizes the translocation of microspheres to various lymph nodes, at time points of 21, 28 and 35 days after administration. Fig. 8 shows the sections of inguinal, brachial and poplitial lymph nodes at 35 days after the sc and id administration of MSs. The translocation rate and extent of the MSs to the lymph nodes was slowest after the sc (single) administration. Reduction in the number of MSs injected per injection site, through sc (multiple) administration, increased the rate and number of MSs translocation. The most rapid translocation of MSs was seen after the multiple id administration. No microspheres were found in the spleen.

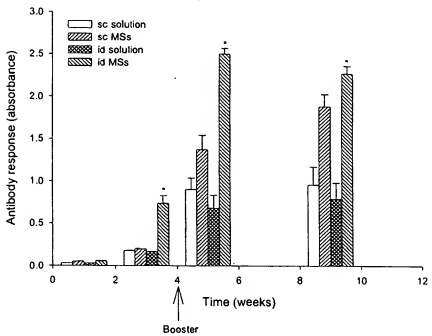

Fig. 4. OVA-specific IgG Ab immune response induced in mice at various time points after immunization with MDP loaded OVA–MSs, injected by sc and id routes. Positive control groups injected with OVA + MDP solution in PBS. Error bars represent ±SEM ($n = 4$ mice for sc and 10 mice for id immunization). * Ab response significantly ($p < 0.05$) different between the two injection routes for the MSs.

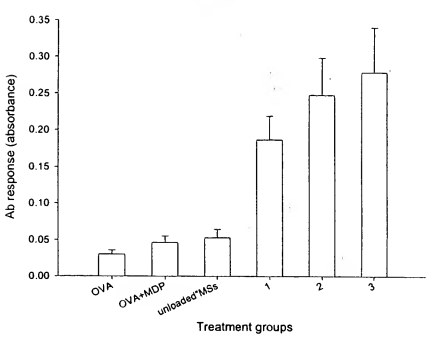

Fig. 5. OVA-specific IgG Ab immune response induced in mice, 4 weeks after id immunization with MDP loaded OVA–MSs: dose–response relationship. Control groups injected with OVA and OVA + MDP solution in PBS and unloaded (non-MDP loaded) OVA–MSs suspended in PBS. Treatment groups injected with MDP loaded OVA–MSs in different doses. Group 1: 700 μg; Group 2: 800 μg; and Group 3: 900 μg (MDP:OVA dose was kept constant). Error bars represent ±SEM (n = 10 mice).

4. Discussion

Upon interstitial administration, a microparticulate vaccine delivery system should drain well from the site of injection, through the interstitial space, and be preferentially taken up into the lymph nodes, where antigen processing and presentation to T-helper cells occurs [44–46]. The uptake and translocation of these microparticles from the site of injection is determined primarily by their size [40,47].

Upon a single sc administration, the fluorescent latex MSs aggregated in the sc tissue (Fig. 1B). The principle barrier to the efficient translocation of the MSs is their drainage from this aggregate through the interstitium and into the initial lymphatics. This resulted in an overall lower number of MSs appearing in the lymph nodes (Table 1 and Fig. 8). A decrease in the number of MSs injected per injection site has been reported to influence the rate of outflow from the administration site [40]. In the present study, it was found that a reduction in the number of MSs per sc injection site, through multiple sc administration of fluorescent MSs led to a diffused distribution of the MSs as compared to a single sc injection. This resulted in a higher number of MSs appearing in various lymph nodes (Table 1 and Fig. 8). Multiple id injections led to a very diffused distribution of MSs in the skin layers of mice, thus increasing the exposed surface

Table 1
Appearance of fluorescent latex microspheres in the lymph nodes (inguinal, brachial and poplitial) and spleen of mice, after administration by sc (single and multiple) and id routes

	Day 21			Day 28			Day 35		
ORGANS	sc (s)[a]	sc (m)[b]	id [c]	sc (s)	sc (m)	id	sc (s)	sc (m)	id
Inguinal	+	+	+ +	+	+ +	+ +	+	+	+
Brachial	+	+	+	+	+	+	+	+ +	+ +
Poplitial	0	+	+	0	+	+ +	+	+ +	+ +
Spleen	0	0	0	0	0	0	0	0	0

[a] sc (s): subcutaneous (single) injection.
[b] sc (m): subcutaneous (multiple) injections.
[c] id: intradermal injections.

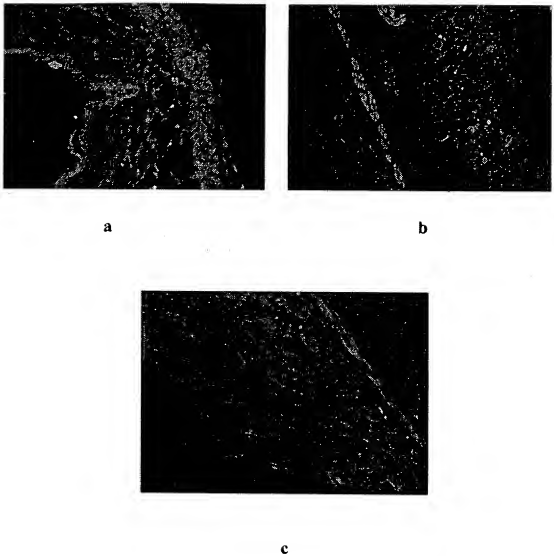

Fig. 6. Skin sections of mice (100×) showing the distribution of id injected fluorescent latex microspheres at (a) 7 days; (b) 14 days; (c) 21 days after administration.

area of the MSs. This increased surface area of the id administered MSs may have led to their efficient translocation to the lymph nodes.

It has been well established that the major pathway of lymphatic uptake for microparticulates is intercellular transport, although some intracellular vesicular transport may also occur [48]. Therefore, it appears that the efficiency of lymph node targeting by the fluorescent MSs was primarily dependent upon their translocation through the interstitial tissue. The route of interstitial administration and the number of MSs injected per injection site were found to be critical factors in modulating translocation efficiency. These observations suggest that the difference in the efficiency of lymph node targeting by the sc and id administered OVA–MSs may explain the higher degree of Ab immune response induced by id injected MSs as compared to sc.

Several authors have reported that the interaction of microparticulates with the APCs can contribute to an enhanced immune response [13,49–51]. The id administered MSs are primarily distributed in the epidermal and dermal layers of the skin. Each of these layers contains numerous immune-competent cells. The epidermis contains Langerhans cells, keratinocytes, dendritic epidermal T lymphocytes, epidermotropic lymphocytes, and melanocytes. The dermis contains lymphocytes, migrant leukocytes, mast cells and tissue macrophages [51]. The epidermal and dermal immune cells, along with an intricate network of cytokines are

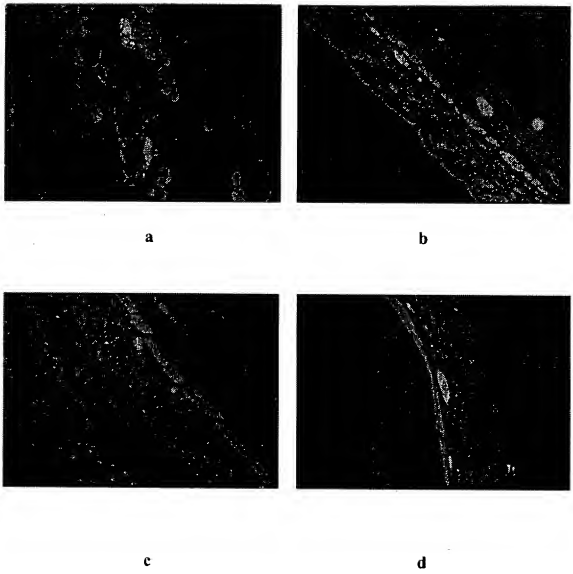

Fig. 7. Skin sections of mice (100×) showing the aggregates of sc (multiple) injected fluorescent latex microspheres at (a) 7 days; (b) 14 days; (c) 28 days; (d) 35 days after administration.

the basis for the cutaneous immune response [52]. Experiments with protein antigens have shown that the density and the state of activation of antigen-presenting macrophages and dendritic cells at the injection site strongly influence the subsequent immune response [13,53–55]. The distribution of the id administered MSs in the epidermis and dermis increases their interaction with the immunological cells and organs. This may lead to efficient phagocytosis of the MSs by macrophages. Microparticulate interactions with macrophages were observed by Nakaoka et al. [56]. The phagocytosis of the MSs results in a direct intracellular delivery of Ag for processing by the major histocompatibility complex (MHC) class II pathway (exogenous), thereby leading to enhanced Ab production [51,57]. Studies by Nakaoka et al. [56] and Kreuter et al. [58] demonstrated that MSs induced macrophage activation leads to the production of IL-1, a cytokine that is strongly associated with Ab production. Therefore, an enhanced interaction of the id administered OVA–MSs with the APCs may also account for the higher Ab response observed following the id immunization, as compared to sc. This was consistent with the observation in this study that an increase in the dosage of MDP loaded OVA–MSs (keeping MDP:OVA dose constant) enhanced the Ab

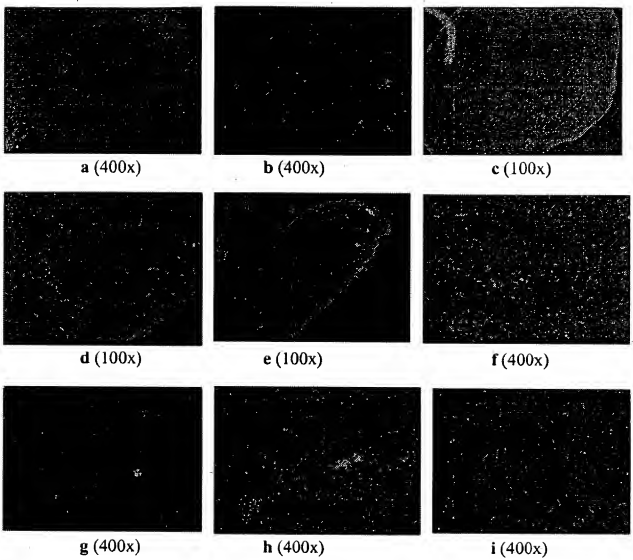

Fig. 8. Lymph node sections of mice, 35 days after administration with fluorescent latex microspheres. (a–c) sc (single) administration; (d–f) sc (multiple) administration; (g–i) id administration. (a, d, g) inguinal lymph node; (b, e, h) brachial lymph node and (c, f, i) popliteal lymph node.

production. This may be due to increased interaction of the larger number of the MSs with the macrophages [22].

5. Conclusions

This study demonstrates that the id route is an effective means of immunization for the microparticulate vaccine delivery systems. As compared to the traditionally used sc route, the id administration of OVA–MSs induced a higher Ab immune response in mice. A decrease in the number of MSs per sc injection site, through multiple sc administration resulted in efficient lymph node targeting as compared to the single sc administration. The most efficient translocation and lymph node targeting was seen from the multiple id administration of MSs. The results of this study suggest that a lower dose of id injected MSs can induce a similar or higher Ab immune response as compared to a higher dose administered sc.

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United States Patent [19]

Srivastava et al.

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[54] **METHOD AND COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE DISEASE USING HEAT SHOCK PROTEINS**

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[22] Filed: **Oct. 16, 1997**

[51] **Int. Cl.**[6] **A61K 39/00**; A61K 39/385; A01N 37/18; C07K 14/435

[52] **U.S. Cl.** **424/193.1**; 424/810; 424/184.1; 514/2; 514/825; 514/866; 514/903; 530/350; 530/806; 530/827; 530/868

[58] **Field of Search** 424/193.1, 810, 424/184.1; 514/2, 866, 903, 825; 530/350, 806, 827, 868

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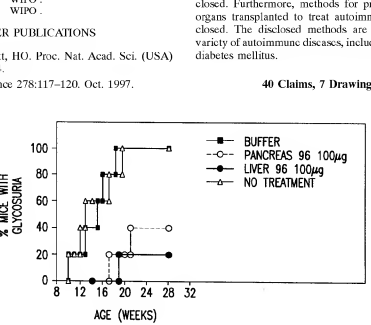
[57] **ABSTRACT**

The invention relates to methods and compositions for the treatment of autoimmune disease. Specifically, compositions comprising heat shock proteins, including gp96, hsp90, and hsp70, are disclosed. Immunotherapeutic methods for administering the hsp-containing compositions are disclosed. Furthermore, methods for preventing rejection of organs transplanted to treat autoimmune disease are disclosed. The disclosed methods are useful for treating a variety of autoimmune diseases, including insulin dependent diabetes mellitus.

40 Claims, 7 Drawing Sheets



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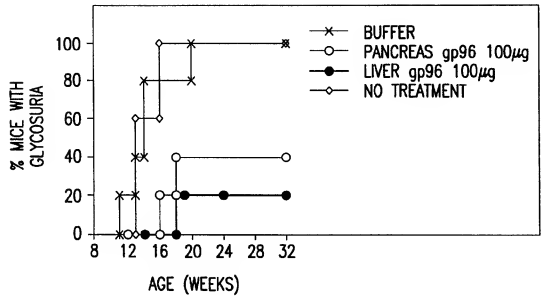
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
BUFFER
PANCREAS gp96 100μg
LIVER gp96 100μg
NO TREATMENT

FIG.1A

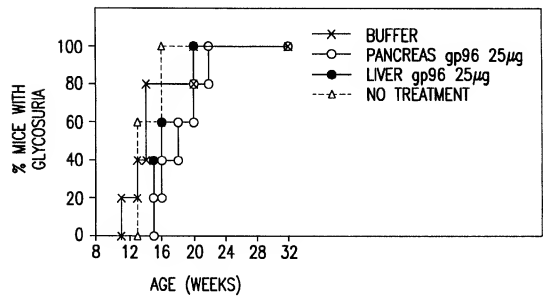
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
BUFFER
PANCREAS gp96 25μg
LIVER gp96 25μg
NO TREATMENT

FIG.1B

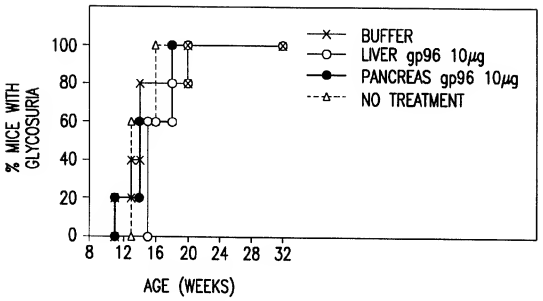
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
BUFFER
LIVER gp96 10μg
PANCREAS gp96 10μg
NO TREATMENT

FIG.1C

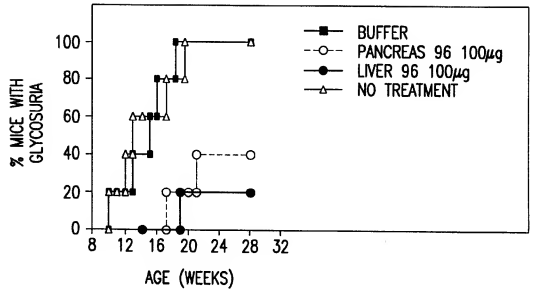
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
BUFFER
PANCREAS 96 100μg
LIVER 96 100μg
NO TREATMENT

FIG.2A

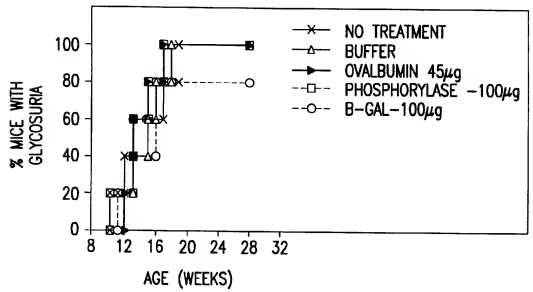
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
NO TREATMENT
BUFFER
OVALBUMIN 45μg
PHOSPHORYLASE -100μg
B-GAL-100μg

FIG.2B

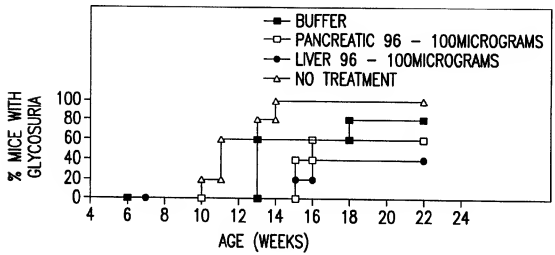
BUFFER
PANCREATIC 96 – 100MICROGRAMS
LIVER 96 – 100MICROGRAMS
NO TREATMENT
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
24
AGE (WEEKS)

FIG. 3A

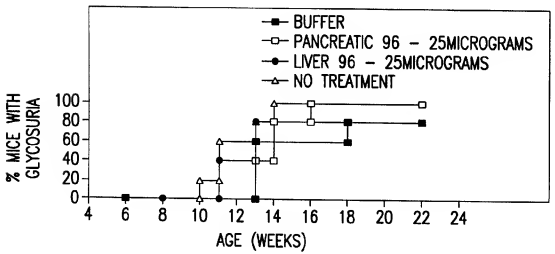
BUFFER
PANCREATIC 96 – 25MICROGRAMS
LIVER 96 – 25MICROGRAMS
NO TREATMENT
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
24
AGE (WEEKS)

FIG. 3B

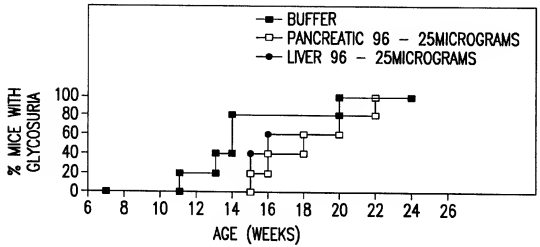
BUFFER
PANCREATIC 96 – 25MICROGRAMS
LIVER 96 – 25MICROGRAMS
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
6 8 10 12 14 16 18 20 22 24 26
AGE (WEEKS)

FIG.3C

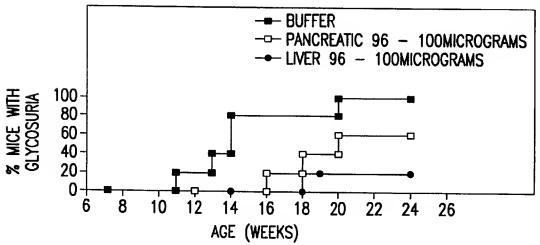
BUFFER
PANCREATIC 96 – 100MICROGRAMS
LIVER 96 – 100MICROGRAMS
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
6 8 10 12 14 16 18 20 22 24 26
AGE (WEEKS)

FIG.3D

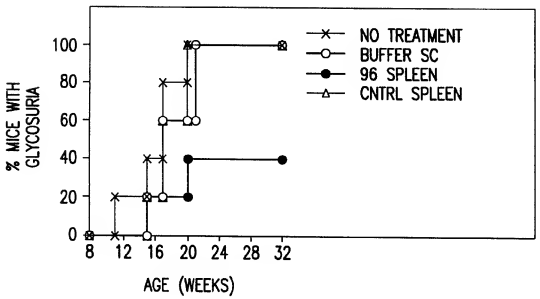
% MICE WITH GLYCOSURIA
100
80
60
40
20
0
8
12
16
20
24
28
32
AGE (WEEKS)
NO TREATMENT
BUFFER SC
96 SPLEEN
CNTRL SPLEEN

FIG.4

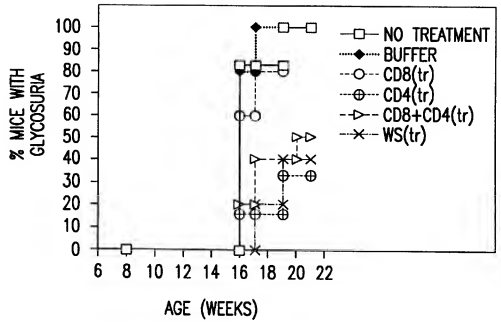
% MICE WITH GLYCOSURIA
100
90
80
70
60
50
40
30
20
10
0
6 8 10 12 14 16 18 20 22
AGE (WEEKS)
NO TREATMENT
BUFFER
CD8(tr)
CD4(tr)
CD8+CD4(tr)
WS(tr)

FIG.5A

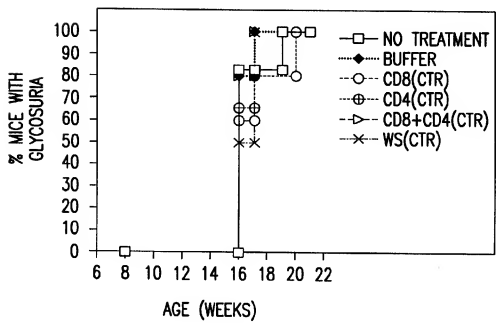
% MICE WITH GLYCOSURIA
100
90
80
70
60
50
40
30
20
10
0
6 8 10 12 14 16 18 20 22
AGE (WEEKS)
NO TREATMENT
BUFFER
CD8(CTR)
CD4(CTR)
CD8+CD4(CTR)
WS(CTR)

FIG. 5B

METHOD AND COMPOSITIONS FOR THE TREATMENT OF AUTOIMMUNE DISEASE USING HEAT SHOCK PROTEINS

This invention was made with government support under grant numbers CA44786 and CA64394 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to methods for treatment of autoimmune disease, including, but not limited to, autoimmune diabetes (i.e., juvenile diabetes or type I diabetes). In the practice of the treatment of autoimmune disease, compositions of complexes of heat shock/stress protein (hsps) including, but not limited to, gp96, hsp90, and hsp70, either alone or in combination with each other, noncovalently bound to antigenic molecules, are used to suppress the autoimmune response. Alternatively, compositions containing un-complexed stress proteins (i.e., free of antigenic molecules) are also used to suppress the immune response.

2. BACKGROUND OF THE INVENTION

Autoimmune diseases result from an abnormal immune response to self antigens. In autoimmune diseases in which the destruction of self tissue results in a metabolic deficiency, some treatment approaches are directed at replacing the deficient element. For example, patients suffering from insulin dependent diabetes mellitus (IDDM) are treated by administering insulin, and pernicious anemia patients are treated with vitamin B_{12}. Even under the best of circumstances, such treatments do not even address, much less reverse, the underlying immunological cause of the deficiency.

Immunological control strategies primarily have been directed either at specific, individual antigens, or at general regulatory processes. As discussed in more detail below, approaches directed at specific antigens first require accurate identification of the offending antigen. Furthermore, many pathological autoimmune responses target more than one single antigen, complicating both the process of antigen identification and the treatment strategy. Also discussed below, immunoregulatory approaches such as treatment with immunosuppressive or anti-inflammatory drugs invariably lead to detrimental effects owing to the systemic influence of the agents on the immune system.

2.1. Immunoregulation in Autoimmune Disease

Insulin is produced in the islets of Langerhans in the pancreas. Human autoimmune type I insulin-dependent diabetes mellitus (IDDM) is characterized by progressive autoimmune destruction of the pancreatic beta-cells in the islets of Langerhans by autoreactive T cells and antibodies. This destructive course is thought to be generated through a break in the peripheral tolerance or a defective clonal deletory mechanism. Non-obese diabetic (NOD) mice are classical murine models which spontaneously develop autoimmune type I IDDM with a similar immunopathological profile to human IDDM (Makino, S., et al., 1985, Current Topics In Clinical And Experimental Aspects of Diabetes (Elsevier: Amsterdam). Development of IDDM in both mice and humans is under polygenic control. IDDM results from CD4, CD8, and macrophage-mediated destruction of pancreatic islet cells (beta cells) (Castano & Eisenbarth, 1990, Ann Rev Immunol 8: 647–79; Haskins et al., 1990, Science 249: 1433–36; Nakano et al., 1991, J. Exp. Med. 173: 1091–7). Destruction of beta cells is mediated by MHC-dependent cytotoxicity. Beta cell auto-antigen specific T cells have been implicated in the pathogenesis of IDDM (Reich et al., 1993, Nature 341: 326–9; Tisch et al., 1993, Nature 366: 72–5; Kaufmen et al., 1993, Nature 366: 69–72). T cell autoreactivity is currently assumed to be due to thymic defect and/or peripheral activation of regulatory T cells secondary to altered production of cytokines (Serreze and Leiter, et al., 1988, J. Immunol. 140: 3801; Serreze et al., 1993, J. Immunol. 150: 2534; Serreze, et al., 1993, Proc. Natl. Acad. Sci. USA 90: 9625; Zipris, et al., 1991, J. Immunol. 146: 3763; Rapoport, et al., 1993, J. Exp. Med. 178: 87).

Susceptibility to IDDM in humans and NOD mice, is strongly linked to the expression of an MHC class II beta chain that lacks the usual aspartic acid residue at position 57 (Asp-57) (Todd, J. A., 1990, Immunol. Today 11: 122–9). In fact, expression of the transgenic class II beta chain containing the Asp-57 protects the NOD mice from spontaneous development of IDDM (Nishimoto et al., 1987, Nature 328:432–4; Bohme, et al., 1990, Science 249:293–5; Miyazaki et al., 1990, Nature 345:722–4; Slattery et al., 1990, Nature 345: 724–6; Singer, et al., 1993, Proc. Natl. Acad. Sci. USA 90:9566–70).

Regulatory T cell-induction has been implicated in resistance to experimentally induced autoimmune encephalitis (EAE) that develops following recovery from an acute episode (Hamaguchi and Leiter, 1990, Diabetes 39: 415; Lider, et al., 1988, Science 239: 181). Tan et al. suggest that the suppressor population is of the Th1 type (Tan et al., 1995, J. Exp. Med. 182: 87–97). However, controversy surrounds the exact type and nature of these cells. Some reports (Boitard, et al., 1989, J. Exp. Med. 169: 1669–1680; Akhtar, et al., 1995, J. Exp. Med. 182. 87–97) assert they are CD4+, whereas others attribute the effect to a wide variety of cloned T cells (Reich, et al., 1989, Diabetes 38: 1647–1651; Pankewycz, et al., 1993, Eur. J. Immunol. 22: 2017–2023; Chosich and Harrison, 1993, Diabetologica 36: 716–721; and Usugi, et al., 1994, Transplantation (Balt) 57: 1799–1804), underscoring the diversity of regulatory T cells in general, and the uncertainty of their potential roles in suppression of autoimmune disease.

Attempts have been made to effect such induction of regulatory T cells through systemic administration of cytokines that mediate a broad-based suppression. Such therapies, however, are too nonspecific and are frequently associated with adverse side-effects.

2.2. Mycobacterial Hsp65 in Autoimmune Arthritis

Adjuvant arthritis in rats is induced by inoculation with various mycobacteria. Such induced arthritis can be suppressed by administration of hsp65 (also referred to as the 64 kD Antigen A) of *Mycobacterium bovis*, i.e. BCG (U.S. Pat. No. 5,354,691; U.S. Pat. No. 5,268,170). BCG is a commonly used adjuvant. BCG hsp65 is identical in amino acid sequence to hsp65 of *Mycobacterium tuberculosis* (Shinnick et al., 1987, Infect. Immun. 55: 1932–1935). U.S. Pat. No. 5,268,170 discloses methods of treatment or prophylaxis of "arthritis-type autoimmune diseases" using this protein.

The human homolog of mycobacterial hsp65 does not appear to be an autoantigen involved in the corresponding human disease, rheumatoid arthritis (in contrast to the role of hsp60 in IDDM discussed in Section 2.3, below). A T-cell epitope in adjuvant arthritis has been identified as the fragment of *Mycobacterium tuberculosis* (or BCG) hsp65 from amino acid 180–188 (see Elias et al., 1991, Proc. Natl.

Acad. Sci. USA 88. 3088–3091, at page 3091, citing van Eden et al., 1988, Nature 331: 171–173). Cross-reactivity between this mycobacterial hsp65 and any self protein apparently results from chance homology between this peptide and some unrelated self protein—since human hsp65 lacks this peptide (see Elias et al., 1991, supra, at page 3091, citing Jindal, et al., 1989, Mol. Cell. Biol. 9: 2279–2283). Accordingly, Elias et al., 1991, supra, reports at page 3091 that rat arthritogenic clone A2b does not respond to human hsp65.

2.3. Heat Shock Proteins As Autoantigens

Self heat shock proteins (hsps) were briefly suggested as possible immunotherapeutic agents against autoimmune disease (International Publication No. WO 89/12455, dated Dec. 28, 1989). This suggestion was based on the precondition that the self stress proteins were the targets of the autoimmune disease in question (International Publication No. WO 89/12455, at page 12, line 7 to page 14, line 23, see especially page 13, lines 11–18). The speculative suggestions contained in International Publication No. WO 89/12455, in view of the lack of practical guidance provided therein, are not instructive for the treatment of autoimmune diseases, either within the context of autoantigen targeted therapy, or as a more general approach.

One particular hsp has been identified as containing a specific autoantigen which is a target of autoimmune IDDM response. The use of *Mycobacterium tuberculosis* hsp65 (also referred to as hsp60) in treating IDDM in NOD mice was reported (Elias et al., 1990, Proc. Natl. Acad. Sci. USA 87: 1576–1580). This report, however, was limited to studying hsp65 as corresponding to a single, unique autoantigen, and not in any way as an immunomodulator. The method was determined not to be applicable to other stress proteins (Elias, et al., 1990, supra, at page 1579).

Further studies were reported using peptide p277, which is a fragment of human hsp60 and has been proposed to contain an epitope corresponding to the key epitope of *M. tuberculosis* hsp65 (U.S. Pat. No. 5,578,303; Elias and Cohen, 1995, Diabetes 44: 1132–1138). U.S. Pat. No. 5,578,303 proposes that the human hsp60 protein "can be used therapeutically" in the treatment of IDDM; no data in which the human hsp60 protein is used is presented (see Example 11, at column 12).

A general problem with attempting to induce tolerance to an autoantigen of a particular disease is that either prior to, or instead of, achieving such tolerance, the administration of the autoantigen may induce the disease by enhancing the destructive immune response against the target tissue. For example, administration of either *M. tuberculosis* hsp65 or p277 can lead to a transient, monophasic hyperglycemia prior to protection (Elias et al., 1990, supra; Elias, et al., 1995, Eur. J. Immunol. 25: 28512857; U.S. Pat. No. 5,578, 303). There is a risk, therefore, of at least a short term exacerbation of disease from such autoantigen administration, making its applicability problematic.

Furthermore, there are at least 12 specific autoantigens and peptides thereof that are targets of IDDM autoimmune response (Solimena and De Camilli, 1996, Nature Medicine 2: 1311). Treatment with p277 alone would not address disorders involving other autoantigens. An effective therapy using peptide autoantigens as immunogens would entail identifying the particular antigen or set of antigens that is the target for a particular IDDM patient.

Thus, the approaches discussed in these studies at best are either too general (e.g., systemic cytokine administration) or too specific (e.g., based on a single autoantigen) to provide practical, effective treatment of autoimmune disease.

Citation or identification of any reference herein shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for the treatment of autoimmune disease. Treatment regimens include the administration of heat shock proteins (hsps), including but not limited to gp96, hsp90, and hsp70. Because the protection is based on the hsp, the effectiveness of the treatment is general—unlike previous approaches, it is not limited to a specific autoantigen or a specific autoimmune disease. The effectiveness of the hsp administration is not dependent on the organ from which the hsp was obtained. Accordingly, the treatment regimens disclosed are useful for the treatment of a variety of autoimmune diseases.

In a preferred embodiment, the treatment regimens provided herein comprise administration of the hsps after the onset of the autoimmune response; i.e., after the specific immune response has already developed. Hsp administration results in regulation of the activity of the relevant, pathologically active effector cells. Thus, the treatment methods of the present invention exploit not only the general properties of hsps but also the specificity of the naturally arisen pathological immune response. Therefore, the treatment methods of the invention are more specific than common cytokine approaches to induction of suppression which are excessively systemic. The hsps used in accordance with the invention exert a more local and targeted transforming effect at the site of autoimmune cellular activity.

In a preferred embodiment, the methods for treatment of autoimmune disease provided herein are directed at reversing the autoimmune response after its onset. Thus, the treatment regimens disclosed herein have the additional advantage over prophylactic methods of being therapeutic. In a preferred embodiment wherein autoimmune diabetes is treated, the hsp is preferably administered no earlier than the period just prior to the onset of glycosuria.

Particular compositions of the invention and their properties are described in the sections and subsections which follow. Optimal doses of hsp administered for treatment of autoimmune disease are provided. In general, the dosages for use in suppressing the immune response are higher than those typically used for generating an immune response. In addition, the invention provides pharmaceutical formulations for administration of the compositions in appropriate dosages. The invention also provides routes of administration of the compositions used for treatment of autoimmune disease.

The example presented in Section 6, below, demonstrates the use of compositions comprising gp96 in the immunotherapeutic treatment of IDDM in a mouse model.

4. BRIEF DESCRIPTION OF THE FIGS.

FIGS. 1A–C. Effect of dosage and tissue origin of gp96 used in immunization on the development of diabetes in non-obese diabetic (NOD) mice. Age of mice in weeks is plotted on the X-axis. Percent of mice with glycosuria is plotted on Y-axis. For each experiment, mice receiving no treatment and treatment with phosphate buffered saline (PBS) alone served as controls. Key: x=PBS alone; ○=Pancreas derived gp96; ●=Liver derived gp96; ◇=No Treatment (FIG. A only); Δ=No treatment (FIGS. 1B and

1C). FIG. 1A: pancreas and liver derived gp96 were each administered in a dose of 100 μg subcutaneously. FIG. 1B: pancreas and liver derived gp96 were each administered in a dose of 25 μg subcutaneously. FIG. 1C: pancreas and liver derived gp96 were each administered in a dose of 10 μg subcutaneously.

FIGS. 2A–B. Specific effectiveness of gp96 immunization as compared with immunization with other proteins. Age of mice in weeks is plotted on the X-axis. Percent of mice with glycosuria is plotted on Y-axis. For each experiment, mice receiving no treatment and treatment with phosphate buffered saline (PBS) alone served as controls. FIG. 2A: liver and pancreas derived gp96 were each administered in a dose of 100 μg. Key for FIG. 2A: Δ=No treatment; ■=PBS alone; ●=Liver derived gp96; ○=Pancreas derived gp96. FIG. 2B: The following non-heat shock proteins were compared to controls: ovalbumin (45 μg dose), phosphorylase-b (100 μg dose), and β-galactosidase (100 μg). Key for FIG. 2B: x=No Treatment; Δ=Buffer alone; ▶=Ovalbumin; □=Phosphorylase-b; ○=galactosidase.

FIGS. 3A–B. Effect of age of animal at time of immunization with gp96. Age of mice in weeks is plotted on the X-axis. Percent of mice with glycosuria is plotted on Y-axis. For each experiment, mice receiving no treatment and treatment with phosphate buffered saline (PBS) alone served as controls. Key: ■=PBS alone; □=Pancreas derived gp96; ●=Liver derived gp96; Δ=No treatment. FIG. 3A: Mice received at 4 weeks of age and immunized at 5 weeks of age. Top graph: pancreas and liver derived gp96 each administered in 100 μg dose. Bottom graph: pancreas and liver derived gp96 each administered in 25 μg dose. FIG. 3B: Mice received at 8 weeks of age and immunized at 8 weeks of age. Top graph: pancreas and liver derived gp96 each administered in 25 μg dose. Bottom graph: pancreas and liver derived gp96 each administered in 100 μg dose.

FIG. 4. Adoptive transfer of gp96-mediated protection against diabetes. Age of mice in weeks is plotted on the X-axis. Percent of mice with glycosuria is plotted on Y-axis. Key: x=No treatment; ○=Buffer SC (0.7 M phosphate buffer used for reconstitution of hsp-peptide complexes); ●=Spleen cells from gp96 treated donor; Δ=Spleen control cells (from pre-diabetic untreated NOD/LtJ mice 6–8 weeks of age).

FIGS. 5A–B. Panel A—Adoptive transfer from mice treated with gp96-peptide complex. Panel B—Adoptive transfer from control mice. Key: □=No treatment; ♦=Buffer SC (0.7 M phosphate buffer used for reconstitution of hsp-peptide complexes); ○=CD8+(CTR); ⊖=CD4+ (CTR); ▷=CD8+ and CD4+ (CTR); x=Spleen control cells (from prediabetic untreated NOD/LtJ mice 6–8 weeks of age).

5. DETAILED DESCRIPTION OF THE INVENTION

Methods and compositions for the treatment of autoimmune disease are described. The invention is based, in part, on newly discovered treatment regimens which provide protection against autoimmune disease. The treatment regimens comprise the administration of hsps, optionally complexed noncovalently with antigenic molecules.

The hsps administered in accordance with the invention are ideal interventional agents against autoimmunity. In specific embodiments, such administered hsps 1) trigger protective immunoregulatory mechanisms effective when administered after the onset of autoimmune damage and, therefore, are immunotherapeutic, as opposed to prophylactic; 2) are effective for long-term protection; 3) effect antigen-specific immune suppression via antigen presenting cells (APCS) which trigger production of suppressor CD4+ Cells that inactivate autoreactive T cells; 4) have a more general effect than individual administered autoantigens, and thus do not require identification of individual autoantigens for effectiveness; 5) nonetheless, have an effect that is specific to at least a substantial portion of the autoantigens that activate autoreactive T cells; 6) are less physiologically disruptive than such non-specific agents as cytokines, because the effect of hsps on the cytokine milieu is mediated through the endogenous, local, cellular response instead of systemically; 7) are of mammalian origin; and 8) are adjuvant free.

Administration of hsps in accordance with the methods described below are a novel immunotherapeutic modality, ideal for the treatment of autoimmune diabetes and other autoimmune diseases as well. In addition, the hsp-based immunotherapeutic methods detailed below are useful for prevention and treatment of rejection of transplanted tissues or organs, such as tissues transplanted to replace those being damaged by autoimmune disease, including but not limited to transplanted islet cells to treat IDDM.

In accordance with the invention, hsps, either uncomplexed or complexed with antigenic molecules, are administered to provide therapeutic treatment or, alternatively, prophylactic protection of autoimmune disease.

"Antigenic molecule" as used herein refers to the peptides with which the hsps are endogenously associated in vivo (e.g., in autoimmune target cells) as well as exogenous antigens/immunogens (i.e., with which the hsps are not complexed in vivo) or antigenic/immunogenic fragments and derivatives thereof.

The hsps of the present invention that can be used include but are not limited to, gp96, hsp90, and hsp70, either alone or in combination with each other. Preferably, the hsps are human hsps.

Heat shock proteins, which are also referred to interchangeably herein as stress proteins, useful in the practice of the instant invention can be selected from among any cellular protein that satisfies any one of the following criteria. A heat shock protein is characterized by having its intracellular concentration increase when a cell is exposed to a stressful stimuli, by being capable of binding other proteins or peptides, by being capable of releasing the bound proteins or peptides in the presence of adenosine triphosphate (ATP) or low pH, or by having at least 35% homology with any cellular protein having any of the above properties.

The first stress proteins to be identified were the heat shock proteins (hsps). As their name implies, hsps are synthesized by a cell in response to heat shock To date, three major families of hsp have been identified based on molecular weight. The families have been called hsp60, hsp70 and hsp90 where the numbers reflect the approximate molecular weight of the stress proteins in kilodaltons. Mammalian hsp90 and gp96 each are members of the hsp90 family. Many members of these families were found subsequently to be induced in response to other stressful stimuli including, but not limited to, nutrient deprivation, metabolic disruption, oxygen radicals, and infection with intracellular pathogens. (See Welch, May 1993, *Scientific American* 56–64; Young, 1990, *Annu. Rev. Immunol.* 8.401–420; Craig, 1993, *Science* 260:19021903; Gething, et al., 1992, *Nature* 355:33–45; and Lindquist, et al., 1988, *Annu. Rev. Genetics* 22:631–677), the disclosures of which are incorporated herein by reference. It is contemplated that hsps/stress proteins belonging to all of these three families can be used in the practice of the instant invention.

The major hsps can accumulate to very high levels in stressed cells, but they occur at low to moderate levels in cells that have not been stressed. For example, the highly inducible mammalian hsp70 is hardly detectable at normal temperatures but becomes one of the most actively synthesized proteins in the cell upon heat shock (Welch, et al., 1985, *J. Cell. Biol.* 101:1198–1211). In contrast, hsp90 and hsp60 proteins are abundant at normal temperatures in most, but not all, mammalian cells and are further induced by heat (Lai, et al., 1984, *Mol. Cell. Biol.* 4:2802–10; van Bergen en Henegouwen, et al., 1987, *Genes Dev.* 1:525–31).

Heat shock proteins are among the most highly conserved proteins in existence. For example, DnaK, the hsp70 from *E. coli* has about 50% amino acid sequence identity with hsp70 proteins from excoriates (Bardwell, et al., 1984, *Proc. Natl. Acad. Sci.* 81:848–852). The hsp60 and hsp90 families also show similarly high levels of intra families conservation (Hickey, et al., 1989, *Mol. Cell. Biol.* 9:2615–2626; Jindal, 1989, *Mol. Cell. Biol.* 9:2279–2283). In addition, it has been discovered that the hsp60, hsp70 and hsp90 families are composed of proteins that are related to the stress proteins in sequence, for example, having greater than 35% amino acid identity, but whose expression levels are not altered by stress. Therefore it is contemplated that the definition of stress protein, as used herein, embraces other proteins, muteins, analogs, or variants thereof having at least 35% to 55%, preferably 55% to 75%, and most preferably 75% to 85% amino acid identity with members of the three families whose expression levels in a cell are enhanced in response to a stressful stimulus. The purification of stress proteins belonging to these three families is described below.

Preferably, the hsp used in accordance with the invention is not a specific autoantigen of the disease which is being treated. In a specific embodiment, the hsp used in accordance with the invention for the treatment of autoimmune disease is not a member of the hsp60 family. In a specific embodiment for the treatment of IDDM, the hsp used in accordance with the invention is not a member of the hsp60 family. In another specific embodiment, e.g. for the treatment of IDDM, the hsp used in accordance with the invention is not hsp65 of *Mycobacterium tuberculosis*, which is identical in sequence to hsp65 of *Mycobacterium bovis*, i.e. BCG (Shinnick et al., 1987, Infect. Immun. 55: 1932–1935). In a specific emobodiment, e.g. for the treatment of arthritis-type autoimmune disease, the hsp used in accordance with the invention is not a member of the hsp60 family. In another specific embodiment, e.g., for the treatment of arthritis-type autoimmune disease, the hsp used in accordance with the invention is not hsp65 of *Mycobacterium tuberculosis*, which is identical in sequence to hsp65 of *Mycobacterium bovis*, i.e. BCG (Shinnick et al., 1987, supra). In yet another specific embodiment, the hsp used in accordance with the invention for the treatment of autoimmune disease is not a mycobacterial protein. In another specific embodiment, the hsp used in accordance with the invention for the treatment of autoimmune disease is not a mycobacterial hsp60. In still another specific embodiment, the hsp used in accordance with the invention is a mammalian hsp.

The immunogenic hsp-peptide complexes of the invention include any complex containing an hsp and a peptide that is capable of inducing an immune response or immunotolerance in a mammal. The peptides are preferably noncovalently associated with the hsp. Preferred complexes include, but are not limited to, hsp90-peptide, hsp70-peptide and hsp60peptide complexes. For example, an hsp called gp96 which is present in the endoplasmic reticulum of eukaryotic cells and is related to the cytoplasmic hsp90's (i.e., is a member of the hsp90 family) can be used to generate an effective vaccine containing a gp96-peptide complex. In a specific embodiment, hsps complexed to the peptides with which they are endogenously associated are used, rather than hsps not so complexed, for purposes of convenience since the endogenous peptides copurify with the hsps.

Although the hsps can be allogeneic to the patient, in a preferred embodiment, the hsps are autologous to (derived from) the patient to whom they are administered. The hsps and/or antigenic molecules can be purified from natural sources, chemically synthesized, or recombinantly produced. The invention provides methods for determining doses for autoimmune disease immunotherapy by evaluating the optimal dose of hsp, both unbound and noncovalently bound to peptide, in experimental tumor models and extrapolating the data.

The therapeutic regimens and pharmaceutical compositions of the invention can be used with additional immune response enhancers or biological response modifiers including, but not limited to, the cytokines IFN-α, IFN-γ, IL-2, IL-4, IL-6, TNT, or other cytokine affecting immune cells. In accordance with this aspect of the invention, the hsp either uncomplexed or complexed with antigenic molecule is administered in combination therapy with one or more of these cytokines.

Accordingly, the invention provides methods of preventing and treating autoimmune disease in an individual comprising administering a composition which elicits specific immunotolerance to the target host cells or tissue.

The invention further relates to administration of compositions comprising hsps, either uncomplexed or complexed to peptides, to individuals at enhanced risk of autoimmune disease due to familial history or environmental risk factors.

5.1. Target Autoimmune Diseases

Autoimmune diseases that can be treated by the methods of the present invention include, but are not limited to, insulin dependent diabetes mellitus (i.e., IDDM, or autoimmune diabetes), multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, ulcerative colitis, and dense deposit disease. The diseases set forth above, as referred to herein, include those exhibited by animal models for such diseases, such as, for example non-obese diabetic (NOD) mice for IDDM and experimental autoimmune encephalomyelitis (EAE) mice for multiple sclerosis.

The methods of the present invention can be used to treat such autoimmune diseases by reducing or eliminating the immune response to the patient's own (self) tissue, or, alternatively, by reducing or eliminating a pre-existing autoimmune response directed at tissues or organs transplanted to replace self tissues or organs damaged by the autoimmune response.

5.2. Obtaining Therapeutic Compositions for Suppression of Autoimmune Response

The hsps used in accordance with the invention can be complexed with antigenic molecules (e.g., peptides), or

uncomplexed. Whether complexed or not, the hsps can be native (non-recombinant) or recombinant. The antigenic molecules can be endogenous, i.e., naturally associated with hsp intracellularly. Alternatively, the antigenic molecules can be exogenous, i.e., not naturally occurring in a noncovalent complex with hsps, or eluted from a cellularly derived noncovalent complex with hsps and reconstituted with other hsps in vitro. Preferably, the hsp, or complex, as the case may be, is used in purified form, preferably to homogeneity as viewed on a polyacrylamide gel, or to at least 60%, 70%, 80%, or 90% of total protein.

The hsp-peptide complexes can be isolated as such from cells wherein the hsp and antigenic molecule are produced. Hsps or exogenous antigenic molecules can be produced in the cell by recombinant expression of a gene encoding that component (either hsp or antigenic molecule), or can be isolated from native sources. The hsps and exogenous antigenic molecule components can be produced and isolated independently and complexed in vitro. Alternatively, complexes of hsps and endogenous peptides can be isolated from cells. In a preferred embodiment for in vitro complexing of hsps and exogenous antigenic molecules, the hsp component is first isolated from cells as a complex, and then purified away from the noncovalently bound endogenous peptide with which it is complexed, prior to complexing in vitro with the exogenous antigenic molecule of interest. Alternatively, the hsp component is first isolated from cells as a complex, and then the noncovalently bound endogenous peptide with which it is complexed is exchanged in vitro with the exogenous antigenic molecule of interest.

Accordingly, the protocols described herein can be used to isolate and produce purified hsps or purified complexes of hsps and antigenic molecules.

Uncomplexed endogenous hsps and endogenous hsps complexed with antigenic molecules can be isolated from any eukaryotic cells, including but not limited to, tissues, isolated cells, and immortalized eukaryotic cell lines. The tissue source need not be the same as the tissue which is targeted by the subject autoimmune response. Suitable source tissues include, but are not limited to liver, or pancreas, or any other organ of mammalian or non-mammalian origin.

Alternatively, the hsps can be produced by recombinant DNA technology using techniques well known in the art. These methods are described in detail in Section 5.2.2, below.

Peptides derived from either a naturally expressed protein (i.e., native peptide) or from a recombinantly expressed protein can be isolated by first isolating the corresponding hsp-peptide complex and then eluting the peptide. Methods for eluting noncovalently bound peptide from the hsp-peptide complex are described in Section 5.2.4, below. Peptides can also be produced synthetically and subsequently complexed with hsps in vitro.

Methods for complexing hsps with antigenic molecules in vitro are described in Section 5.2.5, below.

The hsps to be used therapeutically, alone or complexed, can but need not be isolated from a sample from the patient to which they are then to be administered to treat or prevent autoimmune disorder, i.e., the hsps (and antigenic molecules) can be autologous or non-autologous.

5.2.1. Preparation of Hsp-Peptide Complexes

The methods described in Sections 5.2.1.1–5.2.1.3, below, can be used to isolate hsps complexed with antigenic molecules from cells, preferably from cells expressing non-recombinant hsps, although cells expressing recombinant hsps may also be used. These same methods may also be used to prepare purified hsp, by removing the endogenous antigenic molecules from the isolated complexes by methods described in Section 5.2.3, below.

5.2.1.1. Preparation and Purification of gp96-peptide Complexes

A procedure that can be used, presented by way of example and not limitation, is as follows:

A pellet of eukaryotic cells (e.g., from liver, pancreas, or any other convenient organ) is resuspended in 3 volumes of buffer consisting of 30 mM sodium bicarbonate buffer (pH 7.5) and 1 mM PMSF and the cells allowed to swell on ice 20 minutes. The cell pellet then is homogenized in a Dounce homogenizer (the appropriate clearance of the homogenizer will vary according to each cells type) on ice until >95% cells are lysed.

The lysate is centrifuged at 1,000×g for 10 minutes to remove unbroken cells, nuclei and other debris. The supernatant from this centrifugation step then is recentrifuged at 100,000×g for 90 minutes. The gp96-peptide complex can be purified either from the 100,000×g pellet or from the supernatant.

When purified from the supernatant, the supernatant is diluted with equal volume of 2× lysis buffer and the supernatant mixed for 2–3 hours at 4° C. with Con A-Sepharose® (Pharmacia, Inc., Sweden) equilibrated with PBS containing 2 mM Ca^{2+} and 2 mM Mg^{2+}. Then, the slurry is packed into a column and washed with 1× lysis buffer until the OD_{280} drops to baseline. Then, the column is washed with ⅓ Column bed volume of 10% α-methyl mannoside (α-MM) dissolved in PBS containing 2 mM Ca^{2+} and 2 mM Mg^{2+}, the column sealed with a piece of parafilm, and incubated at 37° C. for 15 minutes. Then the column is cooled to room temperature and the parafilm removed from the bottom of the column. Five column volumes of the α-MM buffer are applied to the column and the eluate analyzed by SDS-PAGE. Typically the resulting material is about 60–95% pure, however this depends upon the cell type and the tissue-to-lysis buffer ratio used. Then the sample is applied to a Mono Q® FPLC ion-exchange chromatographic column (Pharmacia, Inc., Piscataway, N.J.) equilibrated with a buffer containing 5 mM sodium phosphate, pH 7. The proteins then are eluted from the column with a 0–1 M NaCl gradient and the gp96 fraction elutes between 400 mM and 550 mM NaCl.

The procedure, however, can be modified by two additional steps, used either alone or in combination, to consistently produce apparently homogeneous gp96-peptide complexes. One optional step involves an ammonium sulfate precipitation prior to the Con A purification step and the other optional step involves DEAE-Sepharose® purification after the Con A purification step but before the Mono Q® FPLC step.

In the first optional step, the supernatant resulting from the 100,000×g centrifugation step is brought to a final concentration of 50% ammonium sulfate by the addition of ammonium sulfate. The ammonium sulfate is added slowly while gently stirring the solution in a beaker placed in a tray of ice water. The solution is stirred from about ½ to 12 hours at 4° C. and the resulting solution centrifuged at 6,000 rpm (Sorvall SS34 rotor). The supernatant resulting from this step is removed, brought to 70% ammonium sulfate saturation by the addition of ammonium sulfate solution, and centrifuged at 6,000 rpm (Sorvall SS34 rotor). The resulting pellet from this step is harvested and suspended in PBS

containing 70% ammonium sulfate in order to rinse the pellet. This mixture is centrifuged at 6,000 rpm (Sorvall SS34 rotor) and the pellet dissolved in PBS containing 2 mM Ca^{2+} and Mg^{2+}. Undissolved material is removed by a brief centrifugation at 15,000 rpm (Sorvall SS34 rotor). Then, the solution is mixed with Con A Sepharose® and the procedure followed as before.

In the second optional step, the gp96 containing fractions eluted from the Con A column are pooled and the buffer exchanged for 5 mM sodium phosphate buffer, pH 7, 300 mM NaCl by dialysis, or preferably by buffer exchange on a Sephadex® G25 column (Pharmacia, Inc., Sweden). After buffer exchange, the solution is mixed with DEAE-Sepharose® previously equilibrated with 5 mM sodium phosphate buffer, pH 10 7, 300 mM NaCl. The protein solution and the beads are mixed gently for 1 hour and poured into a column. Then, the column is washed with 5 mM sodium phosphate buffer, pH 7, 300 mM NaCl, until the absorbance at 280 nM drops to baseline. Then, the bound protein is eluted from the column with five volumes of 5 mM sodium phosphate buffer, pH 7, 700 mM NaCl. Protein containing fractions are pooled and diluted with 5 mM sodium phosphate buffer, pH 7 in order to lower the salt concentration to 175 mM. The resulting material then is applied to the Mono Q® FPLC column (Pharmacia) equilibrated with 5 mM sodium phosphate buffer, pH 7 and the protein that binds to the Mono Q® FPLC column (Pharmacia) is eluted as described before.

It is appreciated, however, that one skilled in the art can assess, by routine experimentation, the benefit of incorporating the second optional step into the purification protocol. In addition, it is appreciated also that the benefit of adding each of the optional steps will depend upon the source of the starting material.

When the gp96 fraction is isolated from the 100,000xg pellet, the pellet is suspended in 5 volumes of PBS containing either 1% sodium deoxycholate or 1% octyl glucopyranoside (but without the Mg^{2+} and Ca^{2+}) and incubated on ice for 1 hour. The suspension is centrifuged at 20,000xg for 30 minutes and the resulting supernatant dialyzed against several changes of PBS (also without the Mg^{2+} and Ca^{2+}) to remove the detergent. The dialysate is centrifuged at 100,000xg for 90 minutes, the supernatant harvested, and calcium and magnesium are added to the supernatant to give final concentrations of 2 mM, respectively. Then the sample is purified by either the unmodified or the modified method for isolating gp96-peptide complex from the 100,000xg supernatant, see above.

The gp96-peptide complexes can be purified to apparent homogeneity using this procedure. About 10–20 *ug* of gp96peptide complex can be isolated from ig cells/tissue.

5.2.1.2. Preparation and Purification of Hsp 70-peptide Complexes

The purification of hsp70-peptide complexes has been described previously, see, for example, Udono et al., 1993, *J. Exp. Med.* 178:1391–1396. A procedure that can be used, presented by way of example but not limitation, is as follows:

Initially, cells (e.g., from liver, pancreas, or any other convenient organ) are suspended in 3 volumes of 1× lysis buffer consisting of 5 mM sodium phosphate buffer, pH 7, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 1 mM phenyl methyl sulfonyl fluoride (PMSF). Then, the pellet is sonicated, on ice, until >99% cells are lysed as determined by microscopic examination. As an alternative to sonication, the cells can be lysed by mechanical shearing and in this approach the cells typically are resuspended in 30 mM sodium bicarbonate pH 7.5, 1 mM PMSF, incubated on ice for 20 minutes and then homogenized in a dounce homogenizer until >95% cells are lysed.

Then the lysate is centrifuged at 1,000xg for 10 minutes to remove unbroken cells, nuclei and other cellular debris. The resulting supernatant is recentrifuged at 100,000xg for 90 minutes, the supernatant harvested and then mixed with Con A Sepharose® equilibrated with phosphate buffered saline (PBS) containing 2 mM Ca^{2+} and 2 mM Mg^{2+}. When the cells are lysed by mechanical shearing the supernatant is diluted with an equal volume of 2× lysis buffer prior to mixing with Con A Sepharose®. The supernatant is then allowed to bind to the Con A Sepharose® for 2–3 hours at 4° C. The material that fails to bind is harvested and dialyzed for 36 hours (three times, 100 volumes each time) against 10 mM Tris-Acetate pH 7.5, 0.1 mM EDTA, 10 mM NaCl, 1 mM PMSF. Then the dialyzate is centrifuged at 17,000 rpm (Sorvall SS34 rotor) for 20 minutes. Then the resulting supernatant is harvested and applied to a Mono Q® FPLC column equilibrated in 20 mM Tris-Acetate pH 7.5, 20 mM NaCl, 0.1 mM EDTA and 15 mM 2-mercaptoethanol. The column is then developed with a 20 mM to 500 mM NaCl gradient and then eluted fractions fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and characterized by immunoblotting using an appropriate anti-hsp70 antibody (such as from clone N27F3–4, from StressGen, Victoria, British Columbia, Canada).

Fractions strongly immunoreactive with the anti-hsp70 antibody are pooled and the hsp70-peptide complexes precipitated with ammonium sulfate; specifically with a 50%–70% ammonium sulfate cut. The resulting precipitate is then harvested by centrifugation at 17,000 rpm (SS34 Sorvall rotor) and washed with 70% ammonium sulfate. The washed precipitate is then solubilized and any residual ammonium sulfate removed by gel filtration on a Sephadex® G25 column (Pharmacia). If necessary the hsp70 preparation thus obtained can be repurified through the Mono Q® FPLC column as described above.

The hsp70-peptide complex can be purified to apparent homogeneity using this method. Typically 1mg of hsp70peptide complex can be purified from 1g of cells/tissue.

The present invention further describes a rapid method for purification of hsp70-peptide complexes. This improved method comprises contacting cellular proteins with ADP or a nonhydrolyzable analog of ATP affixed to a solid substrate, such that hsp70 in the lysate can bind to the ADP or nonhydrolyzable ATP analog, and eluting the bound hsp70. A preferred method uses column chromatography with ADP affixed to a solid substratum (e.g., ADP-agarose). The resulting hsp70 preparations are higher in purity and devoid of contaminating peptides. The hsp70 yields are also increased significantly by about more than 10 fold. Alternatively, chromatography with nonhydrolyzable analogs of ATP, instead of ADP, can be used for purification of hsp70-peptide complexes.

By way of example but not limitation, purification of hsp70-peptide complexes by ADP-agarose chromatography is carried out as follows:

500 million cells (e.g., from liver, pancreas, or any other convenient organ) are homogenized in hypotonic buffer and the lysate is centrifuged at 100,000xg for 90 minutes at 4° C. The supernatant is applied to an ADP-agarose column.

The column is washed in buffer and is eluted with 5 column volumes of 3 mM ADP. The hsp70-peptide complexes elute in fractions 2 through 10 of the total 15 fractions which elute. The eluted fractions are analyzed by SDS-PAGE. The hsp70-peptide complexes can be purified to apparent homogeneity using this procedure.

5.2.1.3. Preparation and Purification of Hsp 90-peptide Complexes

A procedure that can be used to prepare hsp90-peptide complexes, presented by way of example and not limitation, is as follows:

Initially, cells (e.g., from liver, pancreas, or any other convenient organ) are suspended in 3 volumes of 1× Lysis buffer consisting of 5 mM sodium phosphate buffer (pH7), 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 1 mM phenyl methyl sulfonyl fluoride (PMSF). Then, the pellet is sonicated, on ice, until >99% cells are lysed as determined by microscopic examination. As an alternative to sonication, the cells can be lysed by mechanical shearing and in this approach the cells typically are resuspended in 30 mM sodium bicarbonate pH 7.5, 1 mM PMSF, incubated on ice for 20 minutes and then homogenized in a dounce homogenizer until >95% cells are lysed.

Then the lysate is centrifuged at 1,000×g for 10 minutes to remove unbroken cells, nuclei and other cellular debris. The resulting supernatant is recentrifuged at 1000,000×g for 90 minutes, the supernatant harvested and then mixed with Con A Sepharose® equilibrated with PBS containing 2 mM Ca^{2+} and 2 mM Mg^{2+}. When the cells are lysed by mechanical shearing the supernatant is diluted with an equal volume of 2× Lysis buffer prior to mixing with Con A Sepharose®. The supernatant is then allowed to bind to the Con A Sepharose® for 2–3 hours at 4° C. The material that fails to bind is harvested and dialyzed for 36 hours (three times, 100 volumes each time) against 10 mM Tris-Acetate pH 7.5, 0.1 mM EDTA, 10 mM NaCl, 1 mM PMSF. Then the dialyzate is centrifuged at 17,000 rpm (Sorvall SS34 rotor) for 20 minutes. Then the resulting supernatant is harvested and applied to a Mono Q® FPLC column equilibrated with lysis buffer. The proteins are then eluted with a salt gradient of 200 mM to 600 mM NaCl.

The eluted fractions are fractionated by SDS-PAGE and fractions containing the hsp90-peptide complexes identified by immunoblotting using an anti-hsp90 antibody such as 3G3 (Affinity Bioreagents). Hsp90-peptide complexes can be purified to apparent homogeneity using this procedure. Typically, 150–200 μg of hsp90-peptide complex can be purified from 1 g of cells/tissue.

5.2.2. Recombinant Production of Hsps

Many genes encoding hsps have been cloned and sequenced, including, for example, human hsp70 (GenBank Accession Nos. M11717 and M15432; see also Hunt and Morimoto, 1985, Proc. Natl. Acad. Sci. USA 82: 6455–6459), human hsp90 (GenBank Accession No. X15183; see also Yamazaki et al., 1989, Nucleic Acids Res. 17: 7108), and human gp96 (GenBank Accession No. M33716; see also Maki et al., 1990, Proc. Natl. Acad. Sci. USA 87: 5658–5662).

The hsps can be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing hsp coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, supra, and Ausubel et al., 1989, supra.

A variety of host-expression vector systems can be utilized to express the hsp genes. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the hsp coding sequence; yeast (e.g. Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the hsp coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the hsp coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the hsp coding sequence; or mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the hsp coding sequence can be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101–3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503–5509); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned hsp gene protein can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The hsp gene can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the hsp coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the hsp coding sequence can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing hsps in infected hosts. (See, e.g., Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA

81:3655–3659). Specific initiation signals may also be required for efficient translation of inserted hsp coding sequence. These signals include the ATG initiation codon and adjacent sequences. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516–544).

In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the hsp in the specific fashion desired. For example, choosing a system that allows for appropriate glycosylation is especially important in the case of gp96. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins such as glycosylation. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, etc.

In a preferred embodiment for recombinant expression of hsps, the histidine-nickel (his-Ni) tag system is used (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972–8976). In the his-Ni system, the hsp is expressed in human cell lines as a fusion protein which can be readily purified in a non-denatured form. In this system, the gene of interest (i.e., the hsp gene) is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Kits for expressing an isolating proteins using the his-Ni system are commercially available from Invitrogen®, San Diego, Calif.

Alternatively, recombinant hsps produced in eukaryotic hosts cells as described in this section, above, can be purified according to the respective methods detailed in Section 5.2.1, above.

5.2.3. Preparation and Purification of Uncomplexed hsps

The following methods can be used to obtain uncomplexed hsps, i.e., hsps that are substantially free of noncovalently bound antigenic molecules such as peptides. The hsps can be administered in their uncomplexed form in accordance with the invention for the treatment and prevention of autoimmune disease. In addition, the uncomplexed hsps can be used to design hsp-antigenic molecule complexes by complexing them in vitro with antigenic molecules of interest, as described in Section 5.2.5, below.

5.2.3.1. General Methods

Methods which can be used to separate the hsp and antigenic molecule components of the hsp-antigenic molecule complexes from each other, include, but are not limited to, treatment of the complexes with low pH. The low pH treatment methods described in this section, below, can be used for hsp70, hsp90, or gp96. An alternative method which is preferred for isolating hsp70 from hsp-antigenic molecule complexes is provided in Section 5.2.3.2.

By way of example but not limitation, to elute the noncovalently bound antigenic molecule using low pH, acetic acid or trifluoroacetic acid is added to the purified hsp-antigenic molecule complex to give a final concentration of 10% (vol/vol) and the mixture incubated at room temperature or in a boiling water bath or any temperature in between, for minutes (See, Van Bleek, et al., 1990, *Nature* 348:213–216; and Li, et al., 1993, *EMBO Journal* 12:3143–3151). The resulting samples are centrifuged through a Centricon® 10 assembly. The high and low molecular weight fractions are recovered. The remaining large molecular weight hsp70-peptide complexes can be reincubated in low pH to remove any remaining peptides. The resulting higher molecular weight fractions containing hsp are pooled and concentrated.

5.2.3.2. Preferred Method for Preparation and Purification of Un-complexed Hsp 70

Preferably, the hsp70-peptide complex is purified as described above in Section 5.2.1.2. Once the hsp70-peptide complex is purified, the peptide is eluted from the hsp70 by either of the following two preferred methods. More preferably, the hsp70-peptide complex is incubated in the presence of ATP. Alternatively, the hsp70-peptide complex is incubated in a low pH buffer, as described in Section 5.2.2, above.

Briefly, the complex is centrifuged through a Centricon® assembly (Millipore) to remove any low molecular weight material loosely associated with the complex. The large molecular weight fraction can be removed and analyzed by SDSPAGE while the low molecular weight can be analyzed by HPLC as described below. In the ATP incubation protocol, the stress protein-peptide complex in the large molecular weight fraction is incubated with 10 mM ATP for 30 minutes at room temperature.

The resulting samples are centrifuged through a Centricon® 10 assembly as mentioned previously. The high and low molecular weight fractions are recovered. The remaining large molecular weight hsp70-peptide complexes can be reincubated with ATP to remove any remaining peptides.

The resulting higher molecular weight fractions containing hsp70 are pooled and concentrated.

5.2.4. Isolation of Antigenic Components

The methods described in Section 5.2.3, above, which can be used to isolate hsps from complexes with antigenic molecules, can similarly be used to isolate peptides and/or antigenic components from cells which may contain potentially useful antigenic determinants. Once the hsps and antigenic molecules are separated from each other into separate fractions, the fractions containing the antigenic molecules can be pooled and processed further, as described below. Once isolated, the amino acid sequence of each antigenic peptide can be determined using conventional amino acid sequencing methodologies. Such antigenic molecules can then be produced by chemical synthesis or recombinant methods, purified, and complexed to hsps in vitro.

Similarly, it has been found that potentially immunogenic peptides may be eluted from MHC-peptide complexes using techniques well known in the art (Falk, K. et al., 1990 *Nature* 348:248–251; Elliott, T., et al., 1990, *Nature* 348:195–197; Falk, K., et al., 1991, *Nature* 351:290–296).

Thus, potentially immunogenic or antigenic peptides can be isolated from either stress protein-peptide complexes or

MHC-peptide complexes for use subsequently as antigenic molecules, by complexing in vitro to hsps. Exemplary protocols for isolating peptides and/or antigenic components from either of the these complexes are set forth below in Sections 5.2.4.1 and 5.2.4.2.

5.2.4.1. Peptides From Stress Protein-Peptide Complexes

The methods detailed in Section 5.2.3, above, can be used to elute the peptide from a stress protein-peptide complex. One approach involves incubating the stress protein-peptide complex in the presence of ATP. The other approach involves incubating the complexes in a low pH buffer.

Briefly the complex of interest is centrifuged through a Centricon® 10 assembly (Millipore) to remove any low molecular weight material loosely associated with the complex. The large molecular weight fraction can be removed and analyzed by SDS-PAGE while the low molecular weight can be analyzed by HPLC as described below In the ATP incubation protocol, the stress protein-peptide complex in he large molecular weight fraction is incubated with 10 mM TP for 30 minutes at room temperature. In the low pH protocol, acetic acid or trifluoroacetic acid (TFA) is added to the stress protein-peptide complex to give a final concentration of 10% (vol/vol) and the mixture incubated at room temperature or in a boiling water bath or any temperature in between, for 10 minutes (See, Van Bleek, et al., 1990, *Nature* 348:213–216; and Li, et al., 1993, *EMBO Journal* 12:3143–3151).

The resulting samples are centrifuged through a Centricon® 10 assembly as mentioned previously. The high and low molecular weight fractions are recovered. The remaining large molecular weight stress protein-peptide complexes can be reincubated with ATP or low pH to remove any remaining peptides.

The resulting lower molecular weight fractions are pooled, concentrated by evaporation and dissolved in 0.1% TFA. The dissolved material is then fractionated by reverse phase high pressure liquid chromatography (HPLC) using for example a VYDAC® C18 reverse phase column (Separations Group, Inc., Hesperia, Calif.) equilibrated with 0.1% TFA. The bound material is then eluted at a flow rate of about 0.8 ml/min by developing the column with a linear gradient of 0 to 80% acetonitrile in 0.1% TFA. The elution of the peptides can be monitored by OD_{210} and the fractions containing the peptides collected.

5.2.4.2. Peptides from MHC-peptide Complexes

The isolation of potentially immunogenic peptides from MHC molecules is well known in the art and so is not described in detail herein (See, Falk, et al., 1990, Nature 348:248–251; Rotzschc, at al., 1990, *Nature* 348:252–254; Elliott, et al., 1990, *Nature* 348:191–197; Falk, et al., 1991, *Nature* 351:290–296; Demotz, et al., 1989, *Nature* 343:682–684; Rotzsche, et al., 1990, *Science* 249:283–287, the disclosures of which are incorporated herein by reference).

Briefly, MHC-peptide complexes can be isolated by a conventional immunoaffinity procedure. The peptides then can be eluted from the MHC-peptide complex by incubating the complexes in the presence of about 0.1% TFA in acetonitrile. The eluted peptides can be fractionated and purified by reverse phase HPLC, as before.

The amino acid sequences of the eluted peptides can be determined either by manual or automated amino acid sequencing techniques well known in the art. Once the amino acid sequence of a potentially protective peptide has been determined the peptide can be synthesized in any desired amount using conventional peptide synthesis or other protocols well known in the art.

5.2.4.3. Synthetic Production of Peptides

Peptides having the same amino acid sequence as those isolated above can be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, *J. Am. Chem. Soc.*, 85:2149. During synthesis, N-α-protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its C-terminal and to an insoluble polymeric support i.e., polystyrene beads. The peptides are synthesized by linking an amino group of an N-α-deprotected amino acid to an a-carboxy group of an N-α-protected amino acid that has been activated by reacting it with a reagent such as dicvelohexylcarbodiimide. The attachment of a free amino group to the activated carbuxyl leads to pepiide bond formation. The most commonly used N-a-protecting groups include Boc which is acid labile and Fmoc which is base labile.

Briefly, the C-terminal N-α-protected amino acid is first attached to the polystyrene beads. The N-α-protecting group is then removed. The deprotected α-amino group is coupled to the activated α-carboxylate group of the next N-α-protected amino acid. The process is repeated until the desired peptide is synthesized. The resulting peptides are then cleaved from the insoluble polymer support and the amino acid side chains deprotected. Longer peptides can be derived by condensation of protected peptide fragments. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art and so are not discussed in detail herein (See, Atherton, et al., 1989, *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, and Bodanszky, 1993, *Peptide Chemistry, A Practical Textbook*, 2nd Ed., Springer-Verlag).

Purification of the resulting peptides is accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography The choice of appropriate matrices and buffers are well known in the art and so are not described in detail herein.

5.2.5. In Vitro Production of Stress Protein-Antigenic Molecule Complexes

In an embodiment in which complexes of hsps and the peptides with which they are endogenously associated in vivo are not employed, and it is desired to use hsp-antigenic molecule complexes, complexes of hsps to antigenic molecules are produced in vitro. As will be appreciated by those skilled in the art, the peptides either isolated by the aforementioned procedures or chemically synthesized or recombinantly produced can be reconstituted with a variety of purified natural or recombinant stress proteins in vitro to generate immunogenic noncovalent stress protein-antigenic molecule complexes. Alternatively, exogenous antigens or antigenic/immunogenic fragments or derivatives thereof can be noncovalently complexed to stress proteins for use in the immunotherapeutic or prophylactic vaccines of the invention. A preferred, exemplary protocol for noncovalently complexing a stress protein and an antigenic molecule in vitro is discussed below.

Prior to complexing, the hsps are pretreated with ATP or low pH to remove any peptides that may be associated with the hsp of interest. When the ATP procedure is used, excess

ATP is removed from the preparation by the addition of apyranase as described by Levy, et al., 1991, *Cell* 67:265–274. When the low pH procedure is used, the buffer is readjusted to neutral pH by the addition of pH modifying reagents.

The antigenic molecules (1 μg) and the pretreated hsp (9 μg) are admixed to give an approximately 5 antigenic molecule: 1 stress protein molar ratio. Then, the mixture is incubated for 15 minutes to 3 hours at 4° to 45° C. in a suitable binding buffer such as one containing 20 mM sodium phosphate, pH 7.2, 350 mM NaCl, 3 mM $MgCl_2$ and 1 mM phenyl methyl sulfonyl fluoride (PMSF). The preparations are centrifuged through a Centricon® 10 assembly (Millipore) to remove any unbound peptide. The association of the peptides with the stress proteins can be assayed by SDS-PAGE. This is the preferred method for in vitro complexing of peptides isolated from MHC-peptide complexes of peptides disassociated from endogenous hsp-peptide complexes.

In an alternative embodiment of the invention, preferred for producing complexes of hsp70 to exogenous antigenic molecules such as peptides, 5–10 micrograms of purified hsp is incubated with equimolar quantities of the antigenic molecule in 20 mM sodium phosphate buffer pH 7.5, 0.5 M NaCl, 3 mM $MgCl_2$ and 1 mM ADP in a volume of 100 microliter at 37° C. for 1 hr. This incubation mixture is further diluted to 1 ml in phosphate-buffered saline.

In an alternative embodiment of the invention, preferred for producing complexes of hsp90 to peptides, 5–10 micrograms of purified hsp90 is incubated with equimolar or excess quantities of the antigenic peptide in a suitable buffer such as one containing 20 mM sodium phosphate buffer pH 7.5, 0.5M NaCl, 3 nM $MgCl_2$ at 60–65° C. for 5–20 min. Alternatively, equimolar or excess quantities of peptide (e.g., exogenous peptide) are added to purified hsp90-peptide (endogenous) complex, such that the exogenous peptide is exchanged for the endogenous peptide. In either case, the incubation mixture is allowed to cool to room temperature and centrifuged one or more times if necessary, through a Centricon® 10 assembly (Millipore) to remove any unbound peptide.

In an alternative embodiment of the invention, preferred for producing complexes of gp96 to peptides, 100–300 nM purified peptide is added to 100 nM purified gp96. Alternatively, 100–300 nM peptide (e.g., exogenous peptide) is added to purified gp96-peptide (endogenous) complex, such that the exogenous peptide is exchanged for the endogenous peptide. In either case, the mixture is incubated in a binding buffer consisting of 20 mM HEPES, pH 7.2, 20 mM NaCl, and 2 mM $MgCl_2$ at 60° C. for 10 min. and allowed to cool to room temperature for an additional 10 min. After centrifugation, the sample is incubated for 30 min. at room temperature. Free peptide is removed completely using a microcon 50 (Amicon, Inc.).

Once complexes have been isolated, they can be characterized further for tolerogenicity in animal models using the preferred administration protocols and excipients discussed below.

5.3. Dosage Regimens

Hsps and hsp-antigenic molecule complexes are administered to mammalian subjects, e.g., primates, dogs, cats, mice, rats, horses, cows, pigs, etc., preferably humans, in doses in a range of about 5 μg to about 5000 μg, preferably in a range of about 5 μg to about 1500 μg. In mammals, a range of about 50 μg to about 500 μg, either intradermally or subcutaneously is more preferred, with about 50 μg to about 200 μg subcutaneously and about 5 μg to about 100 μg intradermally even more preferred. Thus, while both subcutaneous and intradermal routes of administration are effective, intradermal injections typically require a lower dosage and are, therefore, preferred with respect to economy of materials. As demonstrated in the example in Section 6, below, an effective dose for treatment of IDDM in the NOD mouse model is 100 μg gp96 subcutaneously or 10 μg gp96 intradermally for mice of average mass of 20–25 g.

Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The hsps or complexes may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local; this may be achieved, for example and not by way of limitation, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In a specific embodiment, the hsp compositions are administered, either intradermally or subcutaneously, with sites of administration varied sequentially. For example, and not by way of limitation, the doses recited above are given once weekly for a period of about 4 to 6 weeks, and the mode of administration is varied with each administration. Each site of administration may be varied sequentially. Thus, by way of example and not limitation, the first injection can be given, either intradermally or subcutaneously, on the left arm, the second on the right arm, the third on the left belly, the fourth on the right belly, the fifth on the left thigh, the sixth on the right thigh, etc. The same site can be repeated after a gap of one or more injections. Also, split injections can be given. Thus, for example, half the dose can be given in one site and the other half in another site on the same day.

After 4–6 weeks, further injections are preferably given at two-week intervals over a period of time of one month. Later injections can be given monthly. The pace of later injections can be modified, depending upon the patient's clinical progress and responsiveness to the immunotherapy. Alternatively, the mode of administration is sequentially varied, e.g., weekly administrations are given in sequence intradermally or subcutaneously.

5.4. Formulation

The uncomplexed hsps or hsps complexed with antigenic molecules, in accordance with the invention, can be formulated into pharmaceutical preparations for administration to mammals, preferably humans, for treatment or prevention of autoimmune diseases. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier can be prepared, packaged, and labelled for treatment of the autoimmune disease, such as insulin dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis,

pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, or dense deposit disease.

If the complex is water-soluble, then it can be formulated in an appropriate buffer, for example, phosphate buffered saline or other physiologically compatible solutions. Alternatively, if the resulting complex has poor solubility in aqueous solvents, then it can be formulated with a non-ionic surfactant such as Tween, or polyethylene glycol. Thus, the compounds and their physiologically acceptable solvates can be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, rectal administration.

For oral administration, the pharmaceutical preparation can be in liquid form, for example, solutions, syrups or suspensions, or can be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets can be coated by methods well-known in the art.

Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds can also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophilic drugs.

The compositions can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

The invention also provides kits for carrying out the therapeutic regimens of the invention. Such kits comprise in one or more containers therapeutically or prophylactically effective amounts of the hsp or hsp-antigenic molecule complexes in pharmaceutically acceptable form. The hsp or hsp-antigenic molecule complex in a vial of a kit of the invention can be in the form of a pharmaceutically acceptable solution, e.g., in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the complex can be lyophilized or desiccated; in this instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (e.g., saline, dextrose solution, etc.), preferably sterile, to reconstitute the complex to form a solution for injection purposes.

In another embodiment, a kit of the invention further comprises a needle or syringe, preferably packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of hsp or hsp-antigenic molecule complexes by a clinician or by the patient.

5.5. Treatment of Autoimmune Disease

The compositions and formulations described above in Sections 5.2 and 5.4 can be used to treat autoimmune disease, including, but are not limited to, insulin dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, ulcerative colitis, and dense deposit disease. Administration of hsp-based compositions can be used to effect tolerance of self tissues and organs that are targets of autoimmune responses, or of tissues transplanted to replace such self target tissues and organs.

5.5.1. Methods of Treatment Based on Administration of Hsps

For treatment of IDDM, the hsps administered in accordance with the invention are most effective in NOD mice when administered just prior to the onset of glycosuria. When the age of onset of glycosuria cannot be predicted, as is

typical with human patients, hsps are preferably administered prior to the onset of islet cell damage once mild glycosuria and mild to moderate levels of hyperglycemia are observed. When administered at this stage, they should provide long term protection against autoimmune disease. Therefore, the methods of treatment of the invention are therapeutic; they protect against the autoimmune response after it has developed. While administration prior to autoimmune response (prophylaxis) is provided by the invention, it is not required and is not as effective as post-response administration.

The example presented in Section 6, below, details the use in accordance with the methods of the invention of the hsp gp96 in immunotherapy in an experimental autoimmune disease model for insulin dependent diabetes mellitus (IDDM). The example presented in Section 7, below, details the use in accordance with the methods of the invention of hsp in immunotherapy in humans for IDDM.

Transplantation is a common form of treatment of autoimmune disease, especially when the target self tissue has been severely damaged. For example, and not by way of limitation, pancreas transplantation and islet cell transplantation are common treatment options for IDDM (See, e.g., Brunicardi, 1996, Transplant. Proc. 28: 2138–40; Kendall & Robertson, 1996, Diabetes Metab. 22: 157–163; Hamano et al., 1996, Kobe J. Med. Sci. 42: 93–104; Larsen & Stratta, 1996, Diabetes Metab. 22: 139–146; and Kinkhabwala, et al., 1996, Am. J. Surg. 171: 516–520). As with any transplantation method, transplantation therapies for autoimmune disease patients include treatments to minimize the risk of host rejection of the transplanted tissue. However, autoimmune disease involves the additional, independent risk that the pre-existing host autoimmune response which damaged the original self tissue will exert the same damaging effect on the transplanted tissue. Accordingly, the present invention encompasses methods and compositions for the treatment of autoimmune disease using heat shock proteins in individuals undergoing transplantation therapy of the autoimmune disease.

In accordance with the invention, the hsp-based compositions and formulations described in Sections 5.2 and 5.4, above, are administered to prevent and treat damage to the transplanted organ, tissue, or cells resulting from the host individual's autoimmune response initially directed against the original self tissue. Preferably, administration is carried out both prior and subsequent to transplantation in 2 to 4 doses each one week apart, at least one of which precedes the transplantation.

5.6. Monitoring of Effects During Autoimmune Disease Immunotherapy

The effects/efficacy of treatment of autoimmune disease according to the present invention can be detected, for example, on the level of the molecular and cellular agents involved in the immune response (e.g., cytotoxic T cells), or on the level of an affected tissue, or on the level of secondary symptoms. In IDDM, one such secondary symptom, glycosuria (i.e., excess sugar in the urine), can be readily assayed to provide an index of the disease state. Accordingly, excess urine in a patient sample as compared with a normal patient sample is symptomatic of IDDM. Efficacy of treatment of such a patient having IDDM is indicated by a resulting decrease in the amount of excess glucose in the urine. In a preferred embodiment for IDDM monitoring, urine samples from patients are assayed for the presence of glucose using techniques well known in the art. Glycosuria in humans is defined by a urinary glucose concentration exceeding 100 mg per 100 ml. Excess sugar levels in those patients exhibiting glycosuria can be measured even more precisely by obtaining blood samples and assaying serum glucose.

6. EXAMPLE: ADMINISTRATION OF GP96 TO AUTOIMMUNE DIABETES MODELS IN MICE

The results described below demonstrate, for the first time, the regulatory function of the hsp gp96 as an inducer of antigen-specific immune suppression. Gp96 vaccination of pre-diabetic Non Obese Diabetic (NOD) mice can elicit long-term protection from diabetes. High doses of hsps, in the presence of an activated T cell population, lead to the production of suppressor population which specifically acts upon the activated T cells. Furthermore, the results below demonstrate that the suppressor cell population elicits protection from autoimmune damage that is long term and can be adoptively transferred.

These results demonstrate that hsps administered in accordance with the invention are ideal interventional agents against autoimmunity. Specifically, such administered hsps 1) trigger protective immunoregulatory mechanisms effective when administered after the onset of autoimmune damage and, therefore, are immunotherapeutic, as opposed to prophylactic; 2) are effective for long-term protection; 3) effect antigen-specific immune suppression via antigen presenting cells (APCs) which trigger production of suppressor CD4+ cells that inactivate autoreactive T cells; 4) have a more general effect than individual administered autoantigens, and thus do not require identification of individual autoantigens for effectiveness; 5) nonetheless, have an effect that is specific to at least a substantial portion of the autoantigens that activate autoreactive T cells; 6) are less physiologically disruptive than such non-specific agents as cytokines, because the effect of hsps on the cytokine milieu is mediated through the endogenous, local, cellular response instead of systemically; 7) are of mammalian origin; and 8) are adjuvant free.

These results indicate that administration of hsps in accordance with the methods described below are a novel immunotherapeutic modality, ideal for the treatment of autoimmune diabetes and other autoimmune diseases as well.

6.1. Materials and Methods

6.1.1. Mice

In vivo assays of efficacy of the immunization regimens were assessed in female NOD/LU mice (commercially available from The Jackson Laboratory, Bar Harbor, Me.). Incidence rates of diabetes in these mice were consistent with those reported in literature, wherein over 80% of female mice developed diabetes by 24 weeks of age and onset of insulitis commenced between 6–8 weeks age. NOD mice are inbred and highly responsive to a variety of immunoregulatory strategies. Adult NOD mice (6–8 weeks of age) have an average mass of 20–25 g.

6.1.2. Gp96 purification

Tissue (liver or pancreas) was obtained from C57/B6 mice (Jackson Laboratory, Bar Harbor, Me.) and processed for purification of hsp-peptide complexes as described earlier (Srivastava et al., 1986, Proc. Natl. Acad. Sci. USA 83: 3407–3411). In all experiments, gp96 was prepared as a complex with endogenous peptide.

6.1.3. Immunization

Gp96-peptide complexes were quantified using spectrometric analysis and appropriate protein quantities were

resuspended prior to injection in 50 μl phosphate buffered saline (PBS) per dose. Two injections, one week apart, were administered subcutaneously under the dorsal skin of each mouse.

6.1.4. Monitoring

Monitoring was performed on two separate occasions prior to immunization and performed weekly throughout the treatment and continued thereafter. Urinc was tested for glucose every eek (Keto-Diastix®; Miles Inc., Kankakee, Ill.) and glycosuric mice were checked for serum glucose (ExacTech®, MediSense, Inc., Waltham, Mass.). Diabetes was diagnosed when fasting glycemia was greater than 2.5g/L.

6.1.5. Adoptive Transfer of Splenic White Cells

Splenic white blood cells were obtained from mice that had been vaccinated intradermally with gp96-peptide complexes at least 5 months prior to their sacrifice and were protected from diabetes in the entire post-immunization period. 5×10^7 splenic white cells obtained from either pre-immunized NOD mice or protected NOD mice were suspended in ^{500} μl PBS and were injected intravenously, retro-orbitally into a fresh batch of 6–8 week-old NOD mice. Similar quantities of cells obtained from non-diabetic NOD mice were injected as controls.

6.1.6. Adoptive Transfer of Fractionated CD4+ and CD8+ Splenic T Cells

Donor NOD mice treated intradermally with 10 μg gp96 complexed with endogenous peptide were parked (i.e., held without further treatment) for 8 weeks and shown to be protected from diabetes. Prediabetic mice, the same age as the recipients, were used as control donors.

Donor mice were sacrificed and their spleen cells were harvested. Red blood cells were removed by lysis by incubating the cell mixture in a filtered solution containing 0.14M ammonium chloride and 0.17M Tris-HCl, pH 7.2. The residual cells were treated with magnetic activated cell sorter (MACS) antibodies reactive with CD4+ and CD8+ cells and loaded onto MACS VS+ Separation Column (Miltenyi Biotec GmbH, Germany). The column containing bound CD4+ and CD8+ cells was washed several times. The cells were eluted off the column by washing the column after removal from the magnetic source and counted. The purity of the CD4+ cells (82% protected mice, 71% control mice) and CD8+ cells (92% protected mice, 67% control mice) was confirmed using fluorescent activated cell sorter (FACS) analysis using a FACScan with Cellquest software (Becton Dickenson, San Jose, Calif.). Cells were resuspended in RPMI-1640 prior to intravenous injection.

Recipient NOD mice were 6–8 weeks old and confirmed non-diabetic at the time of transfer. Untreated recipients or recipients that had received buffer intravenously were used as controls for comparing the degree of protection offered by the adoptive transfer.

Cells were suspended in 200 μl volume of plain RPMI and injected intravenously via the retro-orbital venous plexus. Cell numbers transferred were: 7.5 million CD4+ alone; 4 million CD8+ alone; and 7.5 million CD4+ combined with 4 million CD8+. The cell numbers injected were kept constant irrespective of whether obtained from the treated or the untreated groups of donors.

6.2. Results

In all experiments, each gp96 sample was obtained and used as a complex with endogenous peptide. NOD mice (obtained at 6–8 weeks of age) were immunized at the age of 8 weeks using hsps obtained from sources and in the doses specified: Liver-derived gp96, and pancreas-derived gp96, each in doses of 100 μg (FIG. 1A), 25 μg (FIG. 1B), and 10 μg (FIG. 1C) for each group of five animals. Immunization schedules were as described in Section 6.1.3, above. Control animals were either immunized with PBS-buffer, or were left untreated. As shown in FIGS. 1A–C, gp96-induces long term protection against spontaneous diabetes. Immunization with gp96-peptide complexes derived from mouse liver or pancreas at a dose of 100 μg administered subcutaneously can prevent the spontaneous induction of diabetes in 80% of NOD mice for as long as 8 months as compared to no protection in the untreated group. There is a dose-related response, wherein, lower doses (10 μg subcutaneous) neither delay the onset nor provide protection, intermediate doses (25 μg subcutaneous) delay the onset but do not provide protection, and higher doses (100 μg subcutaneous) provide permanent protection. Lower doses do not hasten the onset of diabetes. Moreover, there was no observed induction or exacerbation of IDDM symptoms in any of the animals as a consequence of gp96 administration.

Unexpectedly, however, there does not appear to be any organ specificity requirement for the source of the gp96: gp96-peptide complexes derived from either liver or pancreas offers protection from diabetes. Furthermore, adjuvant was not used and is, therefore, not required.

In order to determine the specificity of the role of gp96 with respect to other proteins in providing protection, animals immunized with gp96-peptide complexes were compared to control animals immunized with either (i) PBS buffer; (ii) chicken egg albumin (ovalbumin), equivalent of 45 μg/dose; (iii) beta-galactosidase, equivalent of 100 μg/dose; or (iv) phosphorylase-b, equivalent of 100 μg/dose (FIGS. 2A–B). Both, ovalbumin and beta-galactosidase were suspended in PBS and only the supernatant of a post-100,000×g centrifugation was used. This step ensured that only soluble fractions of these proteins were tested, as gp96 is a soluble protein. Mice immunized with the control proteins were unprotected against diabetes and found to develop diabetes at rates comparable to those left untreated.

Therapy of NOD mice 5 weeks of age, using similar doses described above for the 8 week old NOD mice, was also tested. Results indicated that commencement of therapy with gp96-peptide complexes when the mice were 5 weeks old was not as effective as commencement at around 8 weeks of age (FIGS. 3A–B). Vaccination too early (5 weeks) provided less protection from diabetes. This result indicates that gp96 mediated suppression depends on the combined prior appearance of autoantigen(s) and activated T cells reactive against the autoantigen(s).

In order to assess whether protection from diabetes was adoptively transferrable, the following experiment was performed. NOD mice were treated at the age of 8 weeks with liver-derived gp96-peptide complexes or pancreatic-gp96-peptide complexes, each complex in doses of 100 μg per group of animals. All animals were monitored and found free of diabetes for six months following their immunotherapy. Splenic white cells obtained from these animals were adoptively transferred into a fresh batch of 8-week old NOD mice which were normoglycemic at the time of the transfer. Results indicated that adoptive transfer of 5×10^7 splenic white cells/dose given in two doses can protect untreated NOD mice from diabetes, whereas control (pre-diabetic, untreated 6–8 week old NOD/LtJ mice) spleens cannot protect (FIG. 4).

In order to determine which type of T lymphocytes are responsible for the adoptive transfer of suppression of autoimmunity, donor NOD mice were protected from diabetes by treatment intradermally with 10 μg of gp96. Splenic cells were fractionated into CD4+ and CD8+ populations, which were then administered to recipient NOD mice either separately or in combination. As shown in FIGS. **5**A–B, CD4+ cells obtained from protected mice transferred protection from diabetes (FIG. **5**A), whereas controls did not provide protection (FIG. 5B). CD4+ cells alone were most effective, and CD4+ cells in combination with CD8+ cells, as well as whole spleen cells, also conferred protection. CD8+ cells alone did not confer protection.

These results demonstrate that immunization with hsp in accordance with the invention: 1) generates a T cell population that can suppress autoimmune damage; 2) is effective after the onset of insulitis and, therefore, therapeutic; 3) provides protection that can be adoptively transferred; and 4) provides long-term protection.

These results also indicate that gp96 immunotherapy in accordance with the invention is useful for treating diabetic patients undergoing islet cell transplantation. The grafted islet cells of transplant recipients are prone to the same autoreactive damage that originally destroyed the host cells (Verge, et al., 1996, Diabetes 45: 926–933; Tyden, et al., 35 1996, N. Engl. J. Med. 335: 860–863). Immune suppressors specific to only a single autoantigen would be unable to the suppress autoreactive processes directed to a new panoply of transplanted autoantigens for which the recipient suppressor T cell population was hitherto unprimed.

As mentioned above, gp96-mediated protection is most effective when therapy is commenced in mice around 8 weeks of age. Earlier vaccination (5 weeks) provides less effective protection from diabetes. Interestingly, the T-cell response to the autoantigen caused insulitis at around 6–8 weeks. These two events indicate that gp96 is effective in reversing T-cell-mediated beta cell damage, and that gp96 mediated suppression is closely linked to the presence of activated T cells. These results indicate that the optimal period for treatment of humans is at a correspondingly early stage in the development of diabetes, i.e., when mild to moderate glycosuria or hyper glycemia is observed.

Further experiments (data not shown) demonstrated that antigen presenting cells (APCs), upon exposure to hsps, release cytokines such as IFN, and IL-12.

Although the inventors are not required to provide an explanation of the underlying mechanism by which tolerance is effected by the present invention, and without intending to be bound by any one particular mechanistic theory, the following discussion is provided regarding believed mechanisms of the invention. As explained above, gp96-mediated suppression of the pathological immune attack against pancreas cells was not dependent on the source of the gp96 —liver derived gp96, as well as pancreas derived gp96, provided protection. However, the gp96-mediated suppression appears to be dependent on the pre-existing development of the beta-cell-specific autoimmune attack. Thus, the source of gp96 does not require tissue specificity in order to effect suppression because its suppressive activity is only effective against a previously activated T cell response, which is specific. In other words, gp96 suppressive activity is general and, therefore, is not effective, until activated target T cells (which are specifically attacking a self autoantigen) are present, at which time gp96 can suppress that autoimmune T cell activity. Upon exposure to hsps, APCs overproduce a cytokine that activates a regulatory T cell (Tr). When exposed to an activated autoreactive CD8+ T cell (CTL), Tr is transformed into a suppressor T cell (Ts) which inactivates the CTL, effecting protection.

7. EXAMPLE: IMMUNOTHERAPY OF HUMAN IDDM PATIENTS

Immunotherapy is initiated at an early stage in the development of IDDM, preferably when mild glycosuria and/or mild to moderate hyperglycemia are observed. Patients are injected with hsps, either uncomplexed or complexed with peptide, derived from any tissue source, whether autologous, allogeneic, or recombinant. The therapeutic regimen of hsp, for example, gp96, hsp90, hsp70, or a combination thereof, or hsp-peptide complexes containing these hsps, includes weekly injections of the hsp or hsp-peptide complex, dissolved in saline or other physiologically compatible solution. The dosage used is in the range of about 5 μg to about 1500 μg hsp, administered either intradermally or subcutaneously.

The first four to six injections are given at weekly intervals. Subsequently, two injections are given at two-week intervals; followed by a regimen of injections at monthly intervals. The effect of hsp or hsp-peptide complexes therapy is monitored by measuring the amount of glucose present in the urine, or, alternatively, in the serum. Patients suffering from IDDM have excess levels of blood glucose, e.g., 140 mg per 100 ml. Effective treatment is indicated by a reduction in urinary glucose levels toward normal levels, e.g., less than 100 mg per 100 ml.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

What is claimed is:

1. A method of treating autoimmune disease in a mammal comprising administering to the mammal a composition comprising a purified population of complexes consisting essentially of heat shock protein noncovalently bound to a peptide, wherein the heat shock protein is a member of the hsp90 family.

2. The method of claim **1**, wherein the heat shock protein is not an autoantigen of said autoimmune disease.

3. The method of claim **1**, wherein the peptide is not an autoantigen of said autoimmune disease.

4. A method of treating an autoimmune disease in a mammal comprising administering to the mammal a composition comprising a purified heat shock protein which is substantially free of complexed peptide, wherein the heat shock protein is a member of the hsp90 family.

5. The method of claim **4**, wherein the heat shock protein is not an autoantigen of said autoimmune disease.

6. A method of treating autoimmune disease in a mammal comprising administering to the mammal a composition comprising a purified population of complexes consisting essentially of heat shock protein noncovalently bound to a peptide, wherein the heat shock protein is a member of the hsp70 family.

7. The method of claim **6**, wherein the heat shock protein is not an autoantigen of said autoimmune disease.

8. The method of claim **6**, wherein the peptide is not an autoantigen of said autoimmune disease.

9. A method of treating an autoimmune disease in a mammal comprising administering to the mammal a composition comprising a purified heat shock protein which is substantially free of complexed peptide, wherein the heat shock protein is a member of the hsp70 family.

10. The method of claim 6, wherein the heat shock protein is not an autoantigen of said autoimmune disease.

11. The method of claim 1, 4, 2, 3, or 5, wherein the mammal is human.

12. The method of claim 11 wherein the autoimmune disease is insulin dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, ulcerative colitis, or dense deposit disease.

13. The method of claim 1, 4, 2, 3, or 5, wherein the autoimmune disease is insulin dependent diabetes mellitus.

14. The method of claim 13 wherein the heat shock protein is gp96.

15. The method of claim 14 wherein the amount of the heat shock protein present in the composition is in a range of 5 μg to 5000 μg.

16. The method of claim 14 wherein the amount of the heat shock protein present in the composition is in a range of 5 μg to 1500 μg.

17. The method of claim 14 wherein the amount of the heat shock protein present in the composition is in a range of 50 μg to 500 μg.

18. The method of claim 14 wherein the amount of the heat shock protein present in the composition is in a range of 50 μg to 200 μg.

19. The method of claim 18 wherein the composition is administered subcutaneously.

20. The method of claim 14 wherein the amount of the heat shock protein present in the composition is in a range of 5 μg to 100 μg.

21. The method of claim 20 wherein the composition is administered intradermally.

22. The method of claim 1, 4, 2, 3, or 5, further comprising transplanting cells, tissue, or an organ, which correspond to the cells, tissue, or organ affected by the autoimmune disease, from a healthy donor to the mammal being treated.

23. The method of claim 22 wherein the autoimmune disease is insulin dependent diabetes mellitus.

24. The method of claim 23 wherein the mammal is human.

25. The method of claim 23 wherein the heat shock protein is gp96.

26. The method of claim 23 wherein the heat shock protein is hsp90.

27. The method of claim 23 wherein the cells transplanted are pancreatic islet cells.

28. The method of claim 1, 4, 2, 3, or 5, wherein the heat shock protein is hsp90.

29. The method of claim 1, 4, 2, 3, or 5, wherein the disease is multiple sclerosis.

30. The method of claim 29, wherein the hsp is gp96.

31. The method of claim 6, 9, 7, 8, or 10 wherein the mammal is human.

32. The method of claim 31 wherein the autoimmune disease is insulin dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, ulcerative colitis, or dense deposit disease.

33. The method of claim 6, 9, 7, 8, or 10, wherein the autoimmune disease is insulin dependent diabetes mellitus.

34. A method of treating autoimmune disease in a mammal comprising administering to the mammal a composition comprising a purified population of complexes consisting essentially of heat shock protein noncovalently bound to a peptide, wherein the heat shock protein is hsp70, hsp90, or gp96, or a combination of any of the foregoing.

35. The method of claim 32, wherein the peptide is not an autoantigen of said autoimmune disease.

36. The method of claim 34 wherein the mammal is human.

37. The method of claim 34 wherein the autoimmune disease is insulin dependent diabetes mellitus.

38. The method of claim 37 wherein the mammal is human.

39. A method of treating autoimmune disease in a mammal comprising administering to the mammal a composition comprising purified heat shock protein which is substantially free of complexed peptide, wherein the heat shock protein is hsp70, hsp90, or gp96, or a combination of any of the foregoing.

40. The method of claim 32 or 39, wherein the heat shock protein is not an autoantigen of said autoimmune disease.

* * * * *

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: RONALD J. PETTIS, *et al.*	Confirmation No.: 7814
Serial No.: 09/606,909	Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
For: INTRADERMAL DELIVERY OF SUBSTANCES	Attorney Docket No.: 11219-008-999 (500752-999007; P-4901)

DECLARATION OF DR. GERALD B. KASTING
UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Sir:

I, DR. GERALD B. KASTING, do hereby declare and state:

1. I, Gerald B. Kasting, am a citizen of the United States, residing at 44 Mount Pleasant Avenue, Wyoming, Ohio.

2. I received a Ph.D. degree in physical chemistry in 1980 from Massachusetts Institute of Technology in Cambridge, MA under the supervision of Dr. Carl Garland. I was a research chemist at Procter & Gamble Company ("P&G"), from 1980 to 1993. In subsequent years, between 1993 and 1999, I continued as a Senior Scientist at P&G, directing research programs in the Health and Skin Care areas with a particular emphasis on topical and transdermal drug delivery and mathematical modeling of such delivery. From 1999 to the present, I have served as Associate Professor of Pharmaceutics and Cosmetic Science at the

College of Pharmacy in University of Cincinnati, Ohio, and from 2003 to the present, I have served with tenure in the same department.

3. As evidenced by my *curriculum vitae*, appendixed hereto (Exhibit A), I have been working in the field of transdermal and topical delivery of substances as of 1983. The focus of my research has been the analysis of structure-property relationships for delivery of agents through the skin and the development of predictive mathematical models for this phenomenon. Specifically, we have focused on the development of computational models for absorption of substances into and through the skin with the objective of developing better tools for prediction of topical drug delivery, transdermal drug delivery, and dermal exposure to noxious agents. My research group seeks to develop improved techniques for estimating the absorption rates of topically-applied therapeutic agents and for predicting local tissue concentrations and systemic loads resulting from dermal exposure to hazardous agents.

4. My academic background, technical experience and list of publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

5. I am well versed in the biology and physical dynamics of both transdermal and topical delivery of agents, including drugs. I have served as a reviewer for a number of leading peer reviewed journals, including *J. Controlled Rel.*, *Pharm. Res.*, *Int. J. Pharm.*, *J. Invest. Dermatol.*, *Toxicol. Sci.*, *J. Pharm. Sci.*, and *J. Cosmet. Chem.*, and as an editorial board member for the latter two journals. I have published my work as well as various review articles in the field of drug delivery in numerous peer reviewed journals.

6. I have reviewed U.S. Patent Application Serial No. 09/606,909 (the "Pettis Application") which I understand was filed on June 29, 2000. I have also reviewed certain patents and publications that I have been informed were cited during the prosecution of the Pettis Application. More specifically, I have reviewed U.S. Patent Nos. 5,848,991 to Gross *et*

al. ("Gross"); 3,814,097 to Ganderton *et al.* ("Ganderton"); and Autret *et al.*, (1991, *Therapie*, 46:5-8; "Autret"). For purposes of this Declaration, I have been asked to evaluate two issues. First, I have been asked to review the Pettis Application (including its claims, *e.g.*, Claim 29) and comment on the teachings of the Pettis Application and what these teachings would provide to one skilled in the art as of June 2000. Specifically, I have been asked to evaluate whether, by June 2000, a skilled practitioner in the field of drug delivery would, using ordinary skill, be able to successfully deliver drugs with the improved pharmacokinetic profiles claimed following the teachings and guidelines set out in the Pettis Application. Second, I have been asked to comment on the disclosures of Gross, Ganderton and Autret and the distinguishing features, if any, of the teachings of the Pettis Application. I will address these two issues in turn below.

Part I. **Teachings of the Pettis Application**

7. By way of background, scientists in the art of drug delivery are concerned with obtaining systemic distribution of the drug to obtain the needed biological response for a particular therapeutic objective. Systemic distribution can be measured by monitoring plasma drug concentration to determine the pharmacokinetics of the drug -- that is, how the body acts on the drug. Typically, blood samples are taken periodically to measure the plasma concentration over time until the drug has cleared the system. This mode of monitoring was well known and established to those in the art of drug delivery as of the filing date of the Pettis Application -- that is, in June 2000. Practitioners in this art were prepared to routinely conduct pharmacokinetic studies in animal models to characterize the pharmacokinetic profile of a drug. (*See, e.g. The Merck Manual of Diagnosis and Therapy*, 1999, Seventeenth edition, Beers and Berkow, *ed.*, Merck Research Laboratories, Division of Merck & Co., Inc. Whitehouse Station, N.J. (pp. 2559-2567), attached hereto as Exhibit B).

8. To summarize, the Pettis Application describes a methodology for the delivery of drugs to the intradermal compartment of a subject's skin for systemic distribution, using insulin and PTH as an example. According to the Pettis Application, drugs can be delivered intradermally to achieve improved pharmacokinetic profiles when the following critical features are taken into account: (a) accurate positioning and placement of a needle of appropriate length so that its orifice is located within the intradermal compartment (*see,* specification at p. 4, *ll.* 3-28; p. 4 *l.* 29 to p. 5, *l.* 21); and (b) application of pressure in an amount sufficient to control the rate of delivery of the drug (*see,* specification at p. 5, *ll.* 22-28). The method is illustrated by the improved pharmacokinetic profiles achieved for the delivery of insulin and PTH by the intradermal route.

9. A scientist reading the Pettis Application would understand that its teachings are exemplified using insulin and PTH. There is no basis, scientific (or otherwise) to limit the teachings of the specification to insulin and PTH. A scientist armed with the Pettis Application and the technology known to him as of June 2000 would be able to apply the teachings of the invention to other drugs by taking into account the properties of the drug (*e.g.*, the drug's chemical composition, metabolism, and intended use). Moreover, given the compelling results presented in the Pettis Application for insulin, a large molecular weight molecule, a scientist attempting to apply the teachings of the Pettis application to other substances would be optimistic that following the instructions in the Pettis Application would present no impediment to the intradermal delivery of such substances.

10. The Pettis Application teaches that accurate positioning and placement of a needle of appropriate length, shape and structure is the first consideration for achieving intradermal delivery of a drug. The specification provides detailed disclosure regarding the length, shape, form and number of needles that are needed for intradermal delivery (*see,* specification at p. 4, *ll.* 3-28; p. 4 *l.* 29 to p. 5, *l.* 21). The Pettis Application teaches the importance of not only

the needle length but also the shape of the orifice, *e.g.*, exposed height and outlet depth. In the 'Detailed Description of the Invention", the Pettis Application describes the use of microneedles that have *both* a length sufficient to penetrate the intradermal space and an outlet depth within the intradermal space to allow the skin to seal around the needle to prevent effusion of the substance onto the surface of the skin due to backpressure (*see*, specification at p. 5, *ll.* 6-10).

11. The Pettis Application further provides guidance on how to address mechanisms that can be used to provide adequate pressures to control the rate of delivery of the drug so that the drug is consistently delivered to the intradermal compartment of the subject's skin. The Pettis Application clearly sets forth to a scientist reading the application that any of the devices commonly used to generate pressure in an amount sufficient to control the rate of delivery of any drug (*see*, specification at p. 7, *ll.* 25-25, listing for example, pumps, syringes, elastomeric membranes, osmotic pressure, and Belleville springs or washers) may be used in accordance with the invention. A scientist reading the Pettis Application would understand that controlling the application of the pressure is important to prevent the drug from effusing out of the skin which is expected as a result of pressure build up from injection of a substance into a small space.

12. In following the teachings of the Pettis Application, once a scientist has chosen a device having a needle with the appropriate length and shape placing the orifice within the intradermal compartment, the next step would be the application of pressure in an amount effective to control the rate of delivery of a drug for obtaining the desired pharmacokinetic profile. In order to determine the absolute value of the pressure for the delivery of the drug, a scientist would know and expect a certain amount of trial and error experimentation. The scientist would simply assay a series of pressures to arrive at the optimal pressure to achieve the desired pharmacokinetic profile for the drug of his choice. At the onset, a visual

inspection for leakage or excessive weal formation at the site of delivery will allow the scientist to choose a specified pressure as a starting pressure point for intradermal delivery (*see*, the Pettis Application at p. 5, *l.* 22 to p. 6, *l.* 14). The next series of pressures are then chosen in increments above and below the starting point pressure in order to determine the optimal pressure for delivery -- that is the optimal pressure for delivery of a drug for obtaining the desired pharmacokinetic profile. Once the pressure range is chosen, the experimental scheme may comprise the following steps: (1) delivering the drug at a pre-determined pressure within the empirically determined pressure range to the intradermal compartment; (2) taking periodic blood samples to measure the concentration of the drug in the blood until the drug has cleared the system (the number of time points depends on the metabolism of the drug); (3) plotting the blood concentration of the drug over time to obtain a pharmacokinetic profile; and (4) comparing the pharmacokinetic profile to that obtained from subcutaneous delivery of the same drug. If the desired pharmacokinetic profile is not achieved, steps 1-4 will simply be repeated at a different pressure setting. Once the optimal pressure is chosen for intradermal delivery of the drug of choice, the pharmacokinetic profile will be reproducible at that pressure, producing in effect a fingerprint of the drug.

13. Following these teachings of the Pettis Application, a scientist would know to choose a device having a needle so that its orifice is positioned within the intradermal compartment and apply pressure in an amount effective to control the rate of delivery of the drug so that the desired pharmacokinetic profile would be obtained--a pharmacokinetic profile with a higher C_{max} and AUC as compared to delivery of the same drug to the subcutaneous compartment.

14. As I mentioned, such assay schemes requiring the monitoring of drug plasma concentration over a period of time are routinely done in the art of drug delivery to monitor doses of drugs to arrive at accurate dosing regimens for a particular therapeutic effect and

biological response. Thus, even though the amount of experimentation and the time required to carry out such experiments might appear daunting to the lay person, a practitioner in the art of drug delivery would expect it. In fact, when devising experiments in a pre-clinical setting scientists are often cognizant that extrapolation to a clinical setting would require further additional experimentation.

15. Thus, from my perspective, the technology described in the Pettis Application requires a certain amount of experimentation to determine optimal parameters of drug delivery. However, the nature of such experimentation invokes commonly known methodologies and the extent of such experimentation is simply not surprising and is in fact expected.

16. In sum, by June 2000, a skilled practitioner in the art of drug delivery could, using common methodologies known in the art, successfully deliver drugs into the intradermal compartment and achieve improved pharmacokinetic profiles, following the teachings and guidance of the Pettis Application. A scientist would take into account the critical features of the Pettis Application and choose a device having a needle so that its orifice is positioned within the intradermal compartment and apply pressure in an amount effective to control the rate of delivery of the drug so that the desired pharmacokinetic profile would be obtained. Since the scope of the applicability of the teachings of the Pettis Application is not limited to a particular drug, a scientist armed with the Pettis Application and the available technology as of June 2000, could adopt the teachings of the Pettis Application for intradermal delivery of any drug of choice.

Part II. **Relevant Art**

17. I understand that the Pettis Application has been rejected for lacking an inventive contribution over Autret, Ganderton and Gross, purportedly because the cited references disclose the teachings of the Pettis Application. I respectfully disagree and the reasons for my opinion follow.

18. The purported intradermal device and method described in Autret fail to deliver a substance to the intradermal compartment of the subject's skin which is the subject matter of the Pettis Application. In particular, the length and shape of the needle described by Autret is a Lebel single usage needle 4 mm in length with a diameter of 0.4 mm (*see*, Autret at p. 2 of the translation). A needle of such length is outside of the range of needle lengths prescribed by the Pettis Application. Additionally, Autret requires inserting the needle at an angle of approximately 60° from the surface of the subject's skin. Such placement of the needle would result in an angle of about 30° from the normal to the subject's skin, and carrying out basic trigonometric calculation would result in placing the tip of the needle about 3.5 mm deep in to the subject' skin--deeper than the depth taught in the Pettis Application. Thus, despite Autret's characterization of its mesotherapeutic method as "intradermal" the methods described do not result in delivering the substance to the intradermal space as defined in the Pettis Application. In fact, following the method described in Autret, the substance would be delivered non-specifically into the dermis and hypodermis--the fat layer of the skin. The mode of delivery taught in the Pettis Application is thus distinguishable from the Autret method, which results in selective and accurate delivering of the substance to the intradermal compartment of the subjects' skin.

19. Autret is distinguishable from the Pettis Application on other grounds. Autret does not describe the pharmacokinetic profile recited in the claims of the Pettis Application. The pharmacokinetic profile disclosed in Autret (*see*, Autret, Fig. 1) is not improved over

subcutaneous delivery--in fact, the profiles for the two routes of delivery are nearly identical. For example, Autret's pharmacokinetic profile does not exhibit both an increased C_{max} and an increased AUC as required by the claims in the Pettis Application (*e.g.*, Claim 29). While this conclusion is readily apparent from visual inspection of the pharmacokinetic profile, Autret's own characterization of the data supports my position and analysis as well. In the Summary section of the publication, at p. 5, Autret states the following: "[n]either mean plasmatic levels at each plasmatic dosage nor mean areas under the curve....were significantly different" when Autret's method was compared to the subcutaneous route of administration. As summarized by Autret in the Discussion section, "[t]here is *no difference* in one and the same subject between the two routes of administration [intradermal and subcutaneous] as concerns...AUC...*Only* the Cmax is greater with the ID route." *See*, Autret at. p. 10 of the translation. Thus, with respect to pharmacokinetic profiles Autret and the Pettis Application are distinct.

20. Ganderton does not describe an intradermal delivery system which is the subject matter of the Pettis Application. In fact, Ganderton describes a multiple needle array technology, which has in fact been discredited since the late 1990s for failing to achieve a consistent and reproducible mode of drug delivery. The failure of the Ganderton based technology to provide an effective delivery is due, in part, to the inability of closely spaced needles to apply enough pressure to the surface of the skin, except for, at best, puncturing the outer layer of the skin, the stratum corneum. By way of analogy, Ganderton's system is similar to a bed of nails. If there were only one nail, the entire force created by the weight of the body would be distributed over the very small area presented by the tip of the one nail. In this case, the force per unit area, that is, the ratio of the force to the area, would be very great (because the area is small) and would likely result in piercing of the skin. However, when a 'bed' of nails is used, the same force produced by the weight of the body is distributed over

perhaps hundreds of nails. Therefore, the force applied to any one nail is correspondingly reduced, with the result that the force per unit area at the tip of any one nail will be well below the level required to pierce the skin. Since this applies to all the nails in the bed, no nail then penetrates the skin. Similarly, in Ganderton's system since there are multiple fibers the force applied to any one fiber is reduced, resulting in a low force per unit area and at best a puncturing of the outer skin surface, without achieving penetration of any of the fibers into deeper layers of the skin, much less the intradermal space. The multiple fiber pad of Ganderton thus is distinguishable from the intradermal delivery system of the Pettis Application because at best it achieves topical delivery through a punctured stratum corneum.

21. Gross' purported intradermal delivery device is distinct from the intradermal delivery system taught in the Pettis Application. A careful reading of Gross indicates that while there might be some overlap in terms of needle length with the Pettis Application (*see*, Gross at col. 4, *ll.* 10-13) the reference as a whole fails to teach how to specifically deliver a substance into the intradermal compartment as taught in the Pettis Application. Critical features of the Pettis invention are simply absent from Gross. Unlike Gross, the Pettis Application teaches the importance of not only the needle length but also proper positioning of the orifice of the needle into the intradermal compartment of the subject's skin. There is absolutely no disclosure in Gross concerning the height and depth of the needle outlet or the criticality of its placement within the intradermal compartment. Gross does not describe insertion of a needle so that both its outlet depth and exposed height of the outlet (*i.e.*, orifice) are located within the intradermal compartment of the subject's skin. This feature however is a requirement specified in Claim 29 of the Pettis Application and described in the Pettis Application.

22. In fact, even Gross recognizes that its alleged intradermal device does not specifically target the intradermal space. As described in Gross, the Gross devices are designed to administer the drug in a non-selective fashion "below the epidermis, *i.e.*, to the interface

between the epidermis and the dermis *or* to the interior of the dermis *or* subcutaneously" (*see*, Gross at col. 3, *ll.* 39-42; emphasis added). In other words, the devices lack the specificity mandated by those in the Pettis Application--which specifically and selectively deposit a drug into the intradermal compartment of the subject's skin. The delivery systems disclosed in Gross, even though named an intradermal device are by no means specifically targeting the intradermal space. Rather, by Gross' own admission its devices are designed to target various compartments of the skin--each compartment is treated in an equal handed manner. There is thus no disclosure in Gross on how to deliver specifically to one skin compartment to the exclusion of others.

23. The failure of Gross' devices to deliver a substance selectively into the intradermal compartment is, in part, explained by its lack of recognition of proper positioning of the orifice of the needle within the intradermal compartment. If the orifice of the needle (height and depth) is not located within the intradermal space, the application of pressure will not result in ID delivery in accordance with the Pettis Application -- placement of the outlet height above the ID compartment would result in leakage of the injected substance up and out of the injection site; while placement of the outlet depth below the intradermal compartment would result in delivery the subcutaneous compartment.

24. Gross also fails to appreciate the requirement for applying pressure in an amount sufficient to control the rate of delivery of the substance. Gross does not appreciate the shortcomings and limitations associated with gas-pressure driven devices which are prone to deviations in delivery rate as described in the Pettis Application. In fact, Gross treats gas pressure driven devices in the same manner as other pressure generating means. Gross thus provides no teaching of the criticality of the use of mechanical based systems for achieving reproducible and consistent rates of delivery essential for the specificity required for selective ID targeting.

25. Finally, Gross does not describe, measure or evaluate the systemic distribution or pharmacokinetic profile of any substance. More specifically, Gross does not disclose the pharmacokinetic profile required by the claims in the Pettis Application (*e.g.*, Claim 29), *i.e.*, a PK profile with a higher maximum plasma concentration and a higher bioavailability as compared to those obtained from SC delivery. In fact, a careful reading of Gross indicates that Gross does not measure or report plasma concentrations of any drug (*see*, Gross, Figs. 12 and 13).

26. In sum, Gross' failure to disclose the criticality of positioning of the orifice of the needle within the intradermal compartment and application of pressure in an amount sufficient to control the delivery rate of the substance precludes it from selectively targeting a substance to the intradermal compartment and thus achieving the desired PK profile specified in the Pettis claims. In other words, there is simply no guarantee following Gross' disclosure that one would necessarily target the substance to the intradermal compartment--it may happen, it may not.

27. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and that like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States code and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Gerald B. Kasting

Dated: October 6, 2005

GERALD B. KASTING

Express Mail No.: EV 452 774 188 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: RONALD J. PETTIS, et al.	Confirmation No.: 7814
Serial No.: 09/606,909	Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
For: INTRADERMAL DELIVERY OF SUBSTANCES	Attorney Docket No.: 11219-008-999 (500752-999007; P-4901)

DECLARATION OF DR. RONALD J. PETTIS
UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Ronald J. Pettis, do hereby declare and state:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-identified patent application (herein referred to as the '909 application).

2. I am currently a Senior Scientist, at Becton, Dickinson and Company, Inc. which is the assignee of the '909 application.

3. My academic background, technical experience and list of publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

4. I have been asked to comment on how the skilled artisan in the field of drug delivery could use the teachings of the '909 application to successfully deliver drugs with improved pharmacokinetic profiles.

5. By way of background, the pharmacokinetic profile of a drug may be assessed quantitatively by measuring and graphically plotting the serum concentrations of the drug over time. Typically, pharmacokinetic profiles are measured from the start of administration of the drug until the drug is cleared from the bloodstream. Measuring drug serum

concentrations will provide the following parameters: T_{max}, the time required for the drug to reach a maximum serum concentration; C_{max}, the maximum (or peak) serum concentration of the drug reached within a given dose and route of administration; T_{lag} (or "T_{onset}"), the delay time between the administration of a drug and the time for a measurable and detectable blood or plasma level of the drug; and the area under the serum concentration curve (AUC), which is a measure of bioavailability.

6. Pharmacokinetics is traditionally a comparative study, whereby the pharmacokinetic profile of a drug given at different doses or routes of administration may be compared by an inspection of the graphical representation of the profile. Different routes of administration typically include intravenous (IV), intramuscular (IM), and subcutaneous (SC) administration. For example, to achieve maximal C_{max} and bioavailability and minimal T_{max}, ideally drugs are injected directly into the vein, *i.e.*, intravenous administration (IV). However, IV injections are not practical, as they not only must be administered by a health care specialist, they depend on the skill level of that specialist. Intramuscular injections (IM) and subcutaneous injections (SC) have been used traditionally but routinely result in pharmacokinetic parameters that may not be as desirable as those obtained by the IV route. For example, SC or IM delivery typically results in a reduced plasma concentration of the drug, which ultimately has a direct bearing on its efficacy.

7. My co-inventors and I developed an intradermal (ID) drug delivery system that results in an improved pharmacokinetic parameters as compared to SC or IM delivery. This system, as described in the '909 application, involves inserting a needle into the subject's skin so that the needle penetrates the ID compartment and the needle's outlet depth and the exposed height of the outlet are located within the ID compartment. The substance is then delivered through the lumen of the needle by applying pressure in an amount effective to control the rate of delivery of the substance so that improved pharmacokinetic parameters are achieved relative to SC administration. Pressure is applied using any of the commonly known pressure generating devices, such as those disclosed in the '909 application (*e.g.*, pumps, syringes, elastomeric membranes, osmotic pressure or Belleville springs or washers) in an amount effective to control the rate of delivery of the substance. By controlling the rate of delivery of the substance into the ID compartment as described in the '909 application, improved pharmacokinetic parameters may be obtained, as compared to the pharmacokinetic parameters obtained via SC administration of the same substance.

8. As summarized below, the delivery of different substances into the ID compartment in accordance with the teachings of the '909 application results in improved pharmacokinetic parameters, regardless of the mode of application of the pressure (*i.e.* the actual pressure generating device), or the absolute value of the pressure applied, as long as the *rate of delivery was controlled and the needle was placed within the intradermal compartment*. The improved pharmacokinetic profile of the different substances delivered to the ID compartment is manifested as an improvement of two or more of the traditionally measured parameters that characterize the profile, *i.e.*, T_{max}, C_{max} or AUC.

9. The '909 application evidences that delivery of insulin to the ID compartment results in improved pharmacokinetic parameters, *i.e.*, increased C_{max} and AUC, as compared to delivery of insulin to the SC compartment. In Example 2 of the '909 application, insulin was administered to the ID compartment of a pig animal model (*see* the instant specification at page 7, line 25 to page 8, line 21). Insulin was delivered through the lumen of a hollow needle, having a total length of 2 mm, but designed such that the location of the needle outlet was 1 mm--within the ID compartment. Pressure was applied using an infusion pump and the rate of infusion delivery was controlled at a rate of 2U/hr. Blood samples were periodically withdrawn and analyzed for insulin concentration. The results are shown in Figure 4 (attached hereto as Exhibit B), where plasma insulin levels, subsequent to ID administration are plotted over time to generate a serum concentration – time curve (*i.e.,* its pharmacokinetic profile), which is compared to the pharmacokinetic profile observed for SC administration of insulin. It is clear from a visual inspection of Figure 4 that the *pharmacokinetic profile and parameters* of insulin delivered to the ID space is improved relative to that of SC delivery -- *i.e.*, a higher plasma level (increased C_{max}) and a higher bioavailability (increased AUC).

10. In another similar experiment, improved pharmacokinetic parameters were observed when human parathyroid 1-34 (PTH) was delivered to the intradermal compartment using the teachings of the invention. PTH was administered to the intradermal compartment of a pig animal model using a stainless 30 gauge such that the available length for skin penetration was 1-2 mm and the need outlet was at a depth of 1.7-2.0 mm in the skin when the needle was inserted and the total exposed height of the needle was 1-1.2 mm. Flow rate was controlled using a Harvard syringe pump, and PTH was infused for 4 hours at a rate of 75 µl/hr. As shown in Figure 3 of the '909 application (attached hereto as Exhibit C),

delivering PTH to the ID space results in an improved pharmacokinetic profile relative to that obtained by SC administration of PTH, *i.e.*, a faster T_{max}, an increased C_{max} and an increased AUC (the instant specification at p. 7, *ll.* 16-24; and Figure 4).

11. The two examples provided in the '909 application evidence that a substance can be delivered to the ID compartment under sufficient pressure to control the rate of delivery so that a desired pharmacokinetic profile is obtained. The desired profile may be an enhancement in two or more of the following parameters: T_{max}, C_{max} and AUC. In order to apply these teaching to other substances and formulations, one skilled in the art would deliver the substance to the ID compartment via a needle having an appropriate height and outlet depth as specified in the '909 application. One would utilize different pressures to control the rate of delivery. At the onset, a visual inspection of the delivery site to ensure bleb formation and a lack of leakage will be an effective initial indication of delivery to the ID compartment. Subsequent to delivery of the substance, blood samples are periodically drawn and analyzed for concentration of the substance. The results are plotted over time to generate a serum concentration time curve -- the pharmacokinetic profile -- which will provide one skilled in the art with sufficient information to assess if that pressure and rate of delivery are sufficient to provide the desired pharmacokinetic profile. This is indeed what was done in the following examples described in subsequent continuation-in-part applications.

12. For example, in a subsequent continuation-in-part application, U.S. Application Serial No. 09/893,746 ("the '746 application"), filed June 29, 2001, Example 1 shows that ID delivery of insulin results in an improved PK profile as compared to administration via the SC route, *i.e.*, an increased C_{max} and a decreased T_{max}. In Example 1 of the '746 application, insulin was infused in a swine model using a hollow single lumen microneedle (2 mm in length), designed such that the penetration of the needle outlet was limited to 1 mm. The insulin infusion was controlled using an insulin pump wherein the rate of delivery was 2 U/hr for 4 hours. As shown in Figure 1 of the '746 application (attached hereto as Exhibit D), the PK profile shows an increase in higher maximum plasma concentration and a decrease in T_{max} as compared to SC delivery.

13. Example VI of the '746 application shows that delivery of human granulocyte colony stimulating factor (GCSF) to the intradermal compartment of a Yucatan minipig results in an improved pharmacokinetic profile as compared to SC delivery. GCSF was delivered as a bolus using a dermal access design SS3_34 microdevice having a needle length

results in an improved pharmacokinetic profile as compared to SC delivery. GCSF was delivered as a bolus using a dermal access design SS3_34 microdevice having a needle length which places the needle within the ID compartment. Delivery rate was controlled via a Harvard syringe pump. As shown in Figure 6 of the '746 application (attached hereto as Exhibit E), ID delivery of GCSF resulted in a significantly faster T_{max} and a significantly higher AUC as compared to SC delivery (*see* pp. 30-31 of the '746 application, Figure 6).

14. In another continuation-in-part application, U.S. Application No. 10/028,988 ("the '988 application"), filed December 28, 2001, Example VIII shows that delivery of low molecular weight heparin, Fragmin®, to the intradermal compartment of a Yucatan minipig results in an improved pharmacokinetic profile as compared to SC delivery. Fragmin® was delivered as a bolus using a dermal access design SS3_34 microdevice having a needle length which places the needle within the ID compartment. Delivery rate was controlled via hand pressure from a glass microsyringe. As shown in Figure 8 of the '988 application (attached hereto as Exhibit F), ID delivery of Fragmin® resulted in a significantly increased C_{max} and a significantly higher AUC as compared to SC delivery (*see* pp. 32-33 of the '988 application, Figure 8).

15. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and that like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States code and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Dated: Jan 6th, 2005

Ronald J. Pettis

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RONALD J. PETTIS

EDUCATION

1982 - 1986 Georgia Institute of Technology Atlanta, GA
B.S. Chemistry cum laude

1986 - 1988 University of North Carolina Chapel Hill, NC
M.S. Chemistry

1988 - 1991 University of North Carolina Chapel Hill, NC
Ph.D. Chemistry

PROFESSIONAL EXPERIENCE

1996 - Present BD Technologies RTP, NC
Senior Scientist, Team Leader-Therapeutic Drug Delivery
- Wesley J. Howe Award for Corporate Technology Innovation (2001)

1991 - 1996 University of North Carolina, School of Pharmacy Chapel Hill, NC
Research Fellow-Pharmaceutical Formulation and Delivery

PATENTS

6 issued and 17 pending US Patents:

United States Patent 6,722,364 April 20, 2004 *Medicament inhalation delivery devices and methods for using the same*

United States Patent 6,689,100 February 10, 2004 *Microdevice and method of delivering or withdrawing a substance through the skin of an animal*

United States Patent 6,656,147 December 2, 2003 *Method and delivery device for the transdermal administration of a substance*

United States Patent 6,607,513 August 19, 2003 *Device for withdrawing or administering a substance and method of manufacturing a device*

United States Patent 6,595,947 July 22, 2003 *Topical delivery of vaccines*

United States Patent 6,440,096 August 27, 2002 *Microdevice and method of manufacturing a microdevice*

PUBLICATIONS AND PRESENTATIONS

Pettis RJ, Knowles MR, Olivier KN, Kazantseva M, Hickey AJ. *Ionic interaction of amiloride and uridine 5'-triphosphate in nebulizer solutions.* J Pharm Sci. 2004 Sep;93(9):2399-406.

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Pettis, R.J., Hickey, A.J. (1996) *Alveolar macrophage activation by muramyl dipeptide aerosols in guinea pigs*: Effects on cellular morphology, poster, AAPS Southeast Regional Meeting, Research Triangle Park, NC.

Pettis, R.J., Hickey, A.J. (1996) Effect of *muramyl dipeptide aerosols on guinea pig alveolar macrophages*, Pharm. Res., 13(9):S166.

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Mikszta, J. Alarcon, J. M. Brittingham, J. P. Dekker, R. J. Pettis, N. G. Harvey *Microdevice-Based Topical Delivery of DNA and Subunit Vaccines.*

AAPS PharmSci Vol. 2, No. 2, Abstract 2307 (2000)

Pettis, R.J., Haider, I., Mikszta, J., Alarcon, J., Brittingham, J.M., Davison, N., Solbrig, C., Zahn, J. (2001) *Hollow microneedle drug delivery systems: Biomechanical characterization and vaccine delivery*, AAPSPharmSci, Vol. 3, No. 3

Pettis, R.J., Harvey, N., Sutter, D., McFarland, A., Pollack, G., Leipmann, D., Zahn, J. (2002) *Intradermal insulin delivery via MEMS fabricated microneedles*, poster, 2002 AAPS Annual Meeting and Exposition,

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Pettis, RJ, Kaestner, S, Sutter, D, Fentress, J, Harvey, N (2003) *Microneedle-Based Intradermal Delivery of Insulin to Diabetic Swine: A NovelParenteral Administration Method with Unique PK/PD Outcomes*, podium, 2003 CRS Annual Meeting, Honolulu, HA

PROFESSIONAL MEMBERSHIPS AND AFFILIATIONS

Member- Controlled Release Society

Member- American Association of Pharmaceutical Sciences

Member-Editorial Board, Drug Delivery Companies Report

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: RONALD J. PETTIS, et al.	Confirmation No.: 7814
Serial No.: 09/606,909	Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
For: INTRADERMAL DELIVERY OF SUBSTANCES	Attorney Docket No.: 11219-008-999 (500752-999007; P-4901)

SECOND DECLARATION OF DR. RONALD J. PETTIS UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, DR. RONALD J. PETTIS, do hereby declare and state:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-identified patent application (herein referred to as the '909 application).

2. I am currently a Senior Scientist, at Becton, Dickinson and Company, Inc. which is the assignee of the '909 application.

3. My academic background, technical experience and list of publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

4. I have been asked to comment on whether intradermal delivery as practiced in accordance with the methods of the invention would always necessarily result in a higher C_{max} and AUC as compared to subcutaneous delivery as recited in the pending claims of the '909 application.

5. As already described in the Declaration I submitted in connection with the '909 application on January 6, 2005 ("the January Declaration"), my co-inventors and I developed an intradermal (ID) drug delivery system that results in an improved pharmacokinetic profile similar to that observed with subcutaneous (SC) delivery, but with enhanced pharmacokinetic parameters. The improved pharmacokinetic profile can be manifested in two or more of the traditionally measured parameters, *e.g.*, faster T_{max} (the time required for the drug to reach a maximum serum concentration), increased C_{max} (the maximum (or peak) serum concentration of the drug reached within a given dose and route of administration), or increased AUC (the area under the serum concentration curve, which is a measure of bioavailability). The embodiment encompassed by the claims currently pending in the '909 application capture one possible pharmacokinetic outcome-- an increased C_{max} and an increased AUC.

6. However, the injection of a drug to the intradermal comapartment does not inevitably result in an increased C_{max} and an increased AUC. Various factors affect the resultant pharmacokinetic parameters, including the particular substance delivered, the rate of delivery used, and the mode of delivery. When a substance is delivered at a varied rate, pressure, volume or depth, a different pharmacokinetic profile may be obtained as evidenced by the data presented below. In particular, when Genotropin™ was delivered to the intradermal compartment as described in ¶ 8 below, the result was a decreased T_{max} and an increased C_{max} as compared to SC delivery, but with a nearly identical AUC. When Almotriptan was administered to the ID compartment as described in ¶ 10 below, the result was a pharmacokinetic profile nearly identical to SC delivery.

7. In a continuation-in-part application of the '909 application (S. Application Serial No. 10/028,988 ("the '988 application"), filed December 28, 2001), Example XI shows that ID delivery of Genotropin™, a recombinant form of human growth hormone, results in

an improved pharmacokinetic profile as compared to administration via the SC route, *i.e.*, an increased C_{max} and a decreased T_{max}, however, the AUC was nearly identical to that observed with SC delivery.

8. In Example XI of the '988 application, Genotropin™ was delivered in a Yucatan mini pig model using a single microneedle. The microneedle had a total exposed length of 1 mm, designed such that the penetration of the needle outlet was limited to 1 mm. Delivery of the The Genotropin™ was controlled using a syringe pump (Harvard PHD 2000, Harvard Apparatus, Holliston, MA) wherein the rate of delivery was 45 μL/min with a delivery duration of 2.2 minutes. The pharmacokinetic profile for intradermal delivery of Genotropin™ was compared directly to that for subcutaneous injection. The pharmacokinetic parameters of intradermal and subcutaneous delivery of Genotropin™ are summarized in Table 2 of the '988 application and is reproduced below, in part, for convenience.

9. It is clear from an inspection of Table 2 that the *pharmacokinetic profile and pharmacokinetic parameters* for Genotropin™ delivered to the intradermal space is improved relative to that of SC delivery -- *i.e.*, demonstrating a decreased T_{max} and an increased C_{max} -- but does not result in a higher AUC. As shown in Table 2, the most striking observations are the more rapid uptake and distribution associated with ID administration. The measured T_{max} for ID delivery is 4-5 fold more rapid than that seen for SC delivery. ID delivery also exhibits a higher C_{max} resulting from more rapid uptake. The uptake and distribution profiles for ID delivery of Genotropin™ more closely resemble IV, rather than SC administration. However, the calculated AUC values for the ID and SC routes are identical.

PK Parameters	SC	ID Single Needle
Dose (IU/kg)	0.161±0.01	0.164±0.01
C_{max} (mIU/L)	158.5±31.0	612.6±187.1
t_{max} (h)	2.75±0.46	0.47±0.25
$AUC_{INF(pred)}$ (mIU x h/L)	920.2±251.7	850.0±170.0

Table 2: Calculated PK parameters for Genotropin™ administration

10. In Example XII of the '988 application, Axert®, Almotriptan malate ("Almotriptan"), was delivered in a Yucatan mini pig model using a microneedle device. The microneedle had a total exposed length of 1 mm, designed such that the penetration of the needle outlet was limited to 1 mm. The Almotriptan delivery was controlled using a syringe pump (Harvard PHD 2000, Harvard Apparatus, Holliston, MA) wherein the rate of delivery was 45 μL/min and 180 μL/min. The delivery duration was 2-2.5 minutes. The pharmacokinetic parameters of intradermal and subcutaneous delivery of Almotriptan are summarized in Table 3 of the '988 application and reproduced below, in part, for convenience.

PK Parameters	SC	ID
C_{max} (ng/mL)	61.04(19.4)	63.6 (26.1)
t_{max} (h)	0.13(0.05)	0.14(0.08)
AUC	55.9 (6.04)	53.3 (15.7)

Table 3: Almotriptan PK Parameters Following SC and ID Administration

11. It is clear from an inspection of Table 3 that the pharmacokinetic profile and pharmacokinetic parameters of Almotriptan delivered to the intradermal space are similar to SC delivery, but not necessarily enhanced. Indeed, the AUC, C_{max} and T_{max} resulting from intradermal delivery as set out above closely resemble those resulting from SC delivery.

12. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and that like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 19 of the United States code and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Dated: October 6, 2005

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EDUCATION

1982 - 1986 Georgia Institute of Technology Atlanta, GA
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PROFESSIONAL EXPERIENCE

1996 - Present BD Technologies RTP, NC
Senior Scientist, Team Leader-Therapeutic Drug Delivery
- Wesley J. Howe Award for Corporate Technology Innovation (2001)

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Research Fellow-Pharmaceutical Formulation and Delivery

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AAPS PharmSci Vol. 2, No. 2, Abstract 2307 (2000)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.: 09/606,909	Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
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THIRD DECLARATION OF DR. RONALD J. PETTIS
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, DR. RONALD J. PETTIS, do hereby declare and state:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-identified patent application (herein referred to as "the '909 application").

2. I am currently Technology Manager, at Becton, Dickinson and Company, Inc. which is the assignee of the '909 application.

3. My academic background, technical experience and list of publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

4. I have reviewed the claims of the '909 application as amended in the concurrently filed Amendment under 37 C.F.R. § 1.111. I have also reviewed U.S. Patent No. 5,848,991 to Gross *et al.* (herein referred to as "Gross").

5. The presently claimed invention of the '909 application relates to a method for delivering insulin to a specific depth of a human subject's skin through a hollow needle

having an outlet depth and exposed height which are located within the intradermal compartment. The claimed method specifies placement of a needle within the subject's skin so that neither the needle's outlet depth nor its exposed height are outside the intradermal compartment. By using the claimed method, a person of ordinary skill in the art achieves control of the resulting pharmacokinetic profile through intradermal administration of insulin. In contrast, placement of the needle outlet's depth and exposed height outside the intradermal compartment will not result in the instantly claimed higher maximum plasma concentration and higher bioavailability of insulin. Placement of the outlet's depth and exposed height above the intradermal compartment would result in leakage of the injected insulin up and out of the injection site; while placement of the outlet's depth and exposed height below the intradermal compartment would result in delivery to the subcutaneous compartment.

6. The intradermal delivery method claimed in the '909 application is distinct from Gross's purported intradermal delivery method. A careful reading of Gross indicates that while there might be some overlap in terms of needle length with the '909 application (*see*, Gross at col. 4, lines 10-13), the reference as a whole fails to teach how to specifically deliver a substance into the intradermal compartment in order to achieve the improved pharmacokinetic parameters as claimed in the '909 application. Critical features of the invention of the '909 application are simply absent from Gross. Unlike Gross, the '909 application teaches the importance of not only the needle length but also proper positioning of the needle outlet's exposed height into the intradermal compartment of the subject's skin. There is absolutely no disclosure in Gross concerning the height and depth of the needle outlet or the criticality of its placement within the intradermal compartment. Gross does not describe insertion of a needle so that both the depth and exposed height of its outlet (*i.e.*, orifice) are located within the intradermal compartment of the subject's skin. This feature, however, is a requirement specified in Claim 29 of the '909 application. Nor does Gross

disclose the use of intradermal administration as a means of controlling drug pharmacokinetics; further, Gross fails to differentiate intradermal administration from subcutaneous administration.

7. In addition to its effect on insulin's pharmacokinetic profile, further evidence of the significance of accurately placing the needle outlet's depth and exposed height in the intradermal compartment is shown by the outcome of pharmacodynamic studies conducted with insulin. Studies describing the pharmacodynamic response upon administration of insulin to animals in accordance with the claimed invention are described below in paragraphs 8-15.

8. I describe below the results of time course studies of blood glucose concentrations in rabbits upon administration of insulin in accordance with the claimed invention. I designed the studies described herein, which were conducted under my direction, and I am familiar with the results. The studies demonstrate that when insulin is administered in accordance with the claimed method at the same concentration (100 IU/mL) and at the same rate (0.1 mL/h) as described in Exhibit I of Gross (see, col. 10, lines 32-45), a very different pharmacodynamic result is achieved. The different result could only result from a different method of delivery. In fact, as a result of intradermal administration of insulin in accordance via the claimed method at 100 IU/mL, the blood glucose concentrations of the test animals dropped to levels which could not be reversed by ceasing administration of insulin, nor by intervening through administration of glucose to the hypoglycemic test animals. If Gross had been practicing intradermal delivery as claimed in the '909 application, the same result would have been observed; it was not.

9. We administered two different insulin types in the studies. In one study, designated Study 1, we administered to rabbits regular human insulin (Lilly Humulin®). In the other study, designated Study 2, we administered to rabbits a fast acting human insulin

analog (Lilly Lispro®) reported to have a faster onset of, and shorter duration of action than human insulin.

10. We administered a 100 IU/mL solution of insulin at a rate of 0.1 mL/h for two hours. We injected insulin in accordance with the invention at three different depths; at 1 mm, at 2 mm, and at 3 mm. These depths span the range of 0.5-3.0 mm for the penetration depths described at p. 4, lines 5-6 of the '909 application.

11. We injected the rabbits on the right/dorsal flank region using Becton Dickinson single needle catheter sets having a 34 gauge needle. We used a Harvard Apparatus PHD 2000 Program Pump having qualified accuracy for both volume and rate of delivery.

12. In order to further confirm that insulin administered by the method described in paragraphs 10-11 above was injected in the intradermal compartment, in Study 1, we also assessed blood glucose concentrations in a rabbit in which insulin was delivered beneath the surface of the skin into the intradermal compartment using a narrow gauge needle of 1.5 mm length inserted at a shallow angle to the skin to further reduce the depth of administration. We also determined blood glucose concentrations in a rabbit which was not administered with insulin as a negative control in Study 1.

13. For each rabbit in both studies, we analyzed the blood glucose concentrations from blood samples withdrawn from the ear vein using a 25 gauge needle. We sampled the rabbits at 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes. We determined blood glucose concentrations on two different Accuchek Glucose Meters, and averaged the results for each time point.

14. Results - Exhibits B and C present the results of Study 1 (using human insulin), and Exhibits D and E present the results of Study 2 (using the human insulin analog). Exhibits B and D show the average blood glucose levels in mmol/L over the time

course of the studies. Exhibits C and E show the results of the studies, wherein the blood glucose concentrations are normalized to the starting time (time=0) concentration for each rabbit, and thus, present the results obtained for each time point as a percentage of the starting blood glucose concentration.[1] For comparative purposes, the graphs in Exhibits B, C, D, and E superimpose the data reported directly from Figure 12 of Gross.

15. **Results** - All rabbits receiving insulin in the studies experienced more precipitous drops in blood glucose concentrations and lower maximal glucose concentrations than reported in Gross.[2] These observations held for administration of insulin at all three depths (1 mm, 2 mm, and 3 mm) as well as for insulin administered using the 1.5 mm length in Study 1. In fact, all rabbits receiving insulin in the studies had to be humanely euthanized between 2 and 2.5 hours due to seizures caused by severe hypoglycemia, or due to two consecutive blood glucose concentration readings of $\leq$ 10 mg/dL.[3] The severe hypoglycemia experienced by the rabbits could not be reversed by ceasing administration of insulin, nor by administration of glucose. Administration of either type of insulin, *i.e.*, human insulin (in Study 1) and human insulin analog (in Study 2), in accordance with the claimed invention resulted in more precipitous drops in blood glucose concentrations and lower maximal glucose concentrations than reported in Gross.

16. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and that like so made are punishable by fine or imprisonment, or both, under

[1] Presentation of the blood glucose concentration as a percentage change from the starting blood glucose concentration, rather than the absolute blood glucose concentration, facilitates comparison of the blood glucose changes across the population of rabbits used in the study.

[2] Based on tissue site biopsies, the average skin thickness of the rabbits used in the studies was 1.5 mm.

[3] Humane euthanasia endpoints occurred per an institutional animal care and use committee.

Section 1001 of Title 19 of the United States code and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Dated: June 15, 2007

RONALD J. PETTIS

Express Mail No.: EV 654 850 700 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: RONALD J. PETTIS *et al.*	Confirmation No.: 7814
Serial No.: 09/606,909	Group Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
Attorney Docket No.: 11219-008-999 (500752-999007; P-4901)	Date: October 7, 2005

For: INTRADERMAL DELIVERY OF SUBSTANCES

AMENDMENT UNDER 37 CFR § 1.111

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Office Action mailed April 7, 2005 (hereinafter "Office Action"), and pursuant to the provisions of Rule 111, please enter the amendments and consider the remarks below. Applicants submit herewith:

(1) a Petition for Extension of Time for three (3) months from July 7, 2005 up to and including October 7, 2005, with provision for payment of the required extension fee;

(2) an Amendment Fee Transmittal Sheet;

(3) A Second Declaration by Dr. Ronald J. Pettis under 37 C.F.R. §1.132; and

(4) A Declaration by Dr. Gerald Kasting under 37 C.F.R. §1.132.

The Commissioner is hereby authorized to charge any required fee(s) to Jones Day Deposit Account No. 503013.

Listing of Claims begin on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

LISTING OF CLAIMS:

1. (Canceled) A method for delivering a substance into skin comprising delivering the substance into an intradermal space within the skin through a small gauge needle inserted into the intradermal space, wherein an outlet of the needle is inserted at a depth within the skin such that leakage of the substance to the surface of the skin is substantially prevented.

2. (Previously Amended) The method of Claim 29, wherein the needle is selected from the group consisting of microneedles, catheter needles, and injection needles.

3. (Previously Amended) The method of Claim 29, wherein a single needle is inserted.

4. (Previously Amended) The method of Claim 29, wherein multiple needles are inserted.

5. (Previously Amended) The method of Claims 29, wherein the substance is a liquid delivered by pressure directly on the liquid.

6. (Previously Amended) The method of Claims 29, wherein a hormone is delivered.

7. (Previously Amended) The method of Claim 6, wherein the hormone is insulin or PTH.

8. (Canceled) The method of Claim 1 wherein the substance is infused.

9. (Canceled) The method of Claim 1 wherein the substance is injected as a bolus.

10. (Previously Amended) The method of Claim 29, wherein the needle is about 300 μm to 2 mm long.

11. (Previously Amended) The method of Claim 29, wherein the needle is about 500 μm to 1 mm long.

12. (Previously Amended) The method of Claim 29, wherein the outlet is at a depth of about 250 μm to 2 mm when the needle is inserted.

13. (Previously Amended) The method of Claim 29, wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted.

14. (Previously Amended) The method of Claim 29, wherein the outlet has an exposed height of about 0 to 1 mm.

15. (Previously Amended) The method of Claim 29, wherein the outlet has an exposed height of about 0 to 300 μm.

16. (Previously Amended) The method of Claim 29, wherein the delivery rate or volume is controlled by spacing of multiple needles, needle diameter or number of needles.

17. (Withdrawn) A needle for intradermal delivery of a substance into skin comprising means for limiting penetration of the needle into the skin and an outlet positioned such that when the needle is inserted into the skin to a depth determined by the penetration limiting means, leakage of the substance to the surface of the skin is substantially prevented.

18. (Withdrawn) The needle of Claim 17 wherein the outlet is at a depth of about 500 μm to 2 mm when the needle is inserted into the skin.

19. (Withdrawn) The needle of Claim 18 wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted into the skin.

20. (Withdrawn) The needle of Claim 17 which is about 300 μm to 2 mm long.

21. (Withdrawn) The needle of Claim 20 which is about 500 μm to 1 mm long.

22. (Withdrawn) The needle of Claim 17 which is contained in a device comprising a reservoir in fluid communication with the needle.

23. (Withdrawn) The needle of Claim 22 which is contained in a device further comprising pressure-generating means for delivering the substance through the needle.

24. (Withdrawn) The needle of Claim 23 wherein the pressure-generating means provides variable control of substance delivery rate.

25. (Canceled) A method for the administration of a substance to a human subject, comprising delivering the substance into the intradermal compartment of the human subject's skin, so that the substance is distributed systemically.

26. (Canceled) A method for the administration of a substance to a human subject comprising delivering the substance into the intradermal compartment of the human subject's skin in an amount and at a rate sufficient to deliver the substance systemically.

27. (Canceled) A method for the administration of a substance to a human subject, comprising delivering the substance into the intradermal compartment of the human subject's skin, so that a clinically useful amount of the substance is distributed systemically.

28. (Canceled) A method for administration of a substance to a human subject, comprising delivering the substance into an intradermal compartment of the human subject's skin at a controlled volume and rate via a needle having a length sufficient to penetrate the intradermal space and an outlet at a depth within the intradermal space so that the substance is distributed in the plasma.

29. (Currently Amended) A method for administration of a ~~substance~~ drug to a human subject, comprising delivering the ~~substance~~ drug through the lumen of a hollow needle into an intradermal compartment of the human subject's skin, which method comprises

(a) inserting the needle into the subject's skin so that the needle penetrates the intradermal compartment, and the needle's outlet depth and exposed height of the outlet are located within the intradermal compartment, wherein the outlet has an exposed height of about 0 to 1 mm; and

(b) delivering the ~~substance~~ drug through the lumen of the needle with the application of pressure in an amount effective to control the rate of delivery of the ~~substance~~ drug,

so that the ~~substance~~ drug is delivered through the lumen of the needle into the intradermal compartment and distributed systemically exhibiting a pharmacokinetic profile similar to subcutaneous delivery of the ~~substance~~ drug, but with a higher maximum plasma concentration and a higher bioavailability.

30. (Canceled) A method for administration of a substance to a human subject, comprising delivering the substance into an intradermal compartment of the human subject's skin, so that the substance is distributed systemically and has a pharmacokinetic profile similar to subcutaneous delivery of the substance, but with a faster onset of a detectable plasma level.

31. (Canceled) A method for administration of a substance to a human subject, comprising delivering the substance into an intradermal compartment of the human subject's skin, so that the substance is distributed systemically and has a pharmacokinetic profile similar to subcutaneous delivery of the substance, but with a higher bioavailability.

32. (New) The method of claim 29, wherein the drug is used for the treatment of toxicity.

33. (New) The method of claim 32, wherein the drug is an antitoxin.

34. (New) The method of claim 29, wherein the drug is used to control pain.

35. (New) The method of claim 34, wherein the drug is selected from a group consisting of an opioid, an analgesic, or an anesthetic.

36. (New) The method of claim 29, wherein the drug is used to control thrombosis.

37. (New) The method of claim 36, wherein the drug is selected from a group consisting of heparin, coumadin, or warfarin.

38. (New) The method of claim 29, wherein the drug is used to control or eliminate infection.

39. (New) The method of claim 38, wherein the drug is an antibiotic.

REMARKS

After entry of this amendment, claims 2-7, 10-16, 29, and 32-39 will be pending in the application. Claim 29 has been amended to more particularly point out and distinctly claim the invention. New claims 32-39 have been added. The amendments are fully supported by the specification as originally filed and no new matter has been added (*see, e.g.*, p. 6, *ll.* 23-30). Applicants respectfully request that the amendments and remarks made herein be entered into the record of the instant application.

1. THE PENDING CLAIMS SATISFY THE REQUIREMENTS OF 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 29, 2-5, and 10-16 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The rejected claims cover the administration of a drug to the intradermal compartment so that systemic distribution and the specified pharmacokinetic ("PK") profile is achieved.

Although the Examiner concedes that the specification is enabling for delivering insulin, PTH, and similar hormones, he contends that the disclosure is insufficient to support a method of delivering all drugs, at all pressures/flow rates, for any patient. *See*, Office Action at p. 3. Applicants respectfully disagree with this contention, and submit that the Examiner has erroneously focused on the working examples presented in the specification and not the teachings of the specification as a whole.

The Applicants take this opportunity to address how the guidance and working examples of the Pettis Application would enable the skilled artisan to deliver drugs intradermally and achieve the claimed pharmacokinetic profile. In support, the Applicants submit herewith the Declaration of Dr. Kasting ("Kasting Decl."). Dr. Kasting is a Professor

of Pharmaceutics and Cosmetic Sciences, with real-world experience in the field of transdermal drug delivery.

1.1 The Guidance and Working Examples of the Pettis Application Enable the Intradermal Administration of Drugs to Achieve the Claimed Pharmacokinetic Profile

As explained by Dr. Kasting, scientists in the art of drug delivery are concerned with obtaining systemic distribution of drugs in order to obtain the needed biological response for a particular therapeutic objective. Systemic distribution of a drug was (and still is) measured by monitoring the plasma drug concentration to determine the pharmacokinetics of the drug -- *i.e.*, the study of the time course of a drug and its metabolites in the body after administration by any route. For accurate measurement, blood is typically sampled frequently over time until the drug has cleared the system. There was a high level of skill in the art at the time of filing, and practitioners were prepared to conduct such pharmacokinetic studies in animal models to characterize the PK profile of a drug (Kasting Decl., ¶ 7). This is the audience by which enablement of the Pettis Application must be judged.

As shown by Dr. Kasting's analysis, the Pettis Application conveys ample guidance concerning the critical features of the invention, including working examples, that would enable such a scientist using ordinary skill to practice the invention. In particular, the Pettis Application describes the accurate positioning and placement of a needle of appropriate length, shape and structure to target the intradermal space (Kasting Decl., ¶ 10), as well as devices and mechanisms that could be used to apply appropriate pressure to control the rate of delivery through the needle (Kasting Decl., ¶ 11). The guidance does not stop there. The Pettis Application goes on to explain how the practitioner should start -- *e.g.*, by using visual inspection and other real time physical parameters to gauge the right amount of pressure to apply (Kasting Decl., ¶ 12). Given this guidance, as Dr. Kasting points out, the skilled artisan

would be prepared to carry out the routine experiments involved to determine the optimum pressures that can be used to achieve the claimed PK profile. (Kasting Decl., ¶¶ 12, 13 and 14). Once the optimum conditions are selected for delivering a given substance, then it can be applied reproducibly to practice the claimed method and obtain the desired PK profile as recited in claim 29 (Kasting Decl., ¶¶ 12 and 13). Based on the foregoing analysis, Dr. Kasting concludes that a skilled practitioner, using ordinary skill, would be able to successfully deliver drugs with the improved PK profile claimed. (Kasting Decl., ¶¶ 14, 15 and 16). Thus, the claims are enabled.

In the instant case, even by the Examiner's own admission, these routine experimental methods were *well known* to those skilled in the art (*see,* Office Action at p. 2). The mere fact that the blood sampling disclosed in the specification is over a period of 6 hours, and a number of experiments may be needed to arrive at the correct parameters to practice the claimed invention, does not make the experimentation "undue" by the *Wands* standard. *In re Wands*, 858 F.2d 731, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988). Here, as evidenced by Dr. Kasting's declaration, the experimentation required to determine the amount of pressure adequate to control the flow rate of any substance to the intradermal compartment may be done using methods well known in the art. A practitioner in the field of drug delivery would expect to periodically assay, over the course of 6 hours per injection, to determine optimal parameters for drug delivery. The Examiner proffers no basis for his contention that Applicant's data showing about 6 hours of experimentation per pressure value is considered undue in the field of drug delivery. The nature of drug delivery technology is such that it involves conducting a series of assays to determine the optimum conditions needed to provide a desired PK; these include generating a PK profile by measuring blood concentrations of a drug over a period of time, and graphing and analyzing PK profiles. These methods were well known and routinely used by those skilled in the art at the time of

the filing of the instant application. Practitioners of this art were prepared to do this work to arrive at the optimum conditions to be used for drug delivery. (*see*, Kasting Decl., ¶¶ 12 and 14).

Applicants submit that as emphasized in *Wands*, in the standard test for what constitutes "undue experimentation" the key word is "*undue*", not "experimentation". It appears, that the Examiner has based the enablement rejection on the necessity for *some* experimentation -- but, according to the legal standard, this is permissible. In fact a "*considerable* amount of experimentation is permissible, *if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.*" *In re Wands*, 858 F.2d at 737, citing *In re Jackson*, 217 U.S.P.Q. 804, 807 (Bd. App. 1982) (emphasis added). Experimentation by nature requires a certain amount of trial and error -- but experimentation alone is not considered "undue" so long as the methodology is *routine*. *In re Angstadt*, 190 U.S.P.Q. 214 (C.C.P.A. 1976).

1.2 The Premises For Non-Enablement Are Unfounded

The Examiner deems the specification enabling with respect to insulin, PTH and similar hormones, yet arbitrarily contends that the disclosure is not enabling with respect to other substances. The Examiner's arbitrary line drawing is unsupported. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re Fisher*, 427 F.2d 833, 839, 166 U.S.P.Q. 18, 24 (C.C.P.A. 1970). Here, as evidenced by Dr. Kasting's Declaration the teachings of the specification, exemplified by the delivery of insulin and PTH, are sufficient guidance for one

skilled in the art to apply to other substances to achieve the claimed PK profile. (Kasting Decl., ¶¶ 8 and 9).

The Examiner also cites unpredictability of biological systems, particularly with respect to drug effects on individuals, as evidence that one skilled in the art would not be able to make and use the claimed methods of the invention. However, the particular physiological effect of a drug on the human body is not relevant to the enablement of the pending claims -- which are directed to the distribution of the drug in the body. The Examiner has conflated pharmacodynamics, *i.e.*, how the drug acts on the body, with pharmacokinetics, *i.e.*, how the body acts on the drug. The latter is the subject matter of the claimed invention. (*See*, Amendment filed July 10, 2003 for a detailed discussion). Thus, unpredictability associated with the effect of a drug's actions on a body, as cited by the Examiner, is not relevant to the PK profile of a drug. PK profiles are routinely used to evaluate optimum doses, formulations, and routes of administration. In fact, a given drug, when delivered in the same dose and route of administration, has a reproducible "fingerprint" PK profile; *see*, Kasting Decl., ¶ 12. Thus, the unpredictability of biological systems alleged by the Examiner as a factor, is not applicable to determining enablement of the claimed method.

The Examiner's point concerning Applicant's own "uncertainty" (Office Action, p. 3, 2d paragraph, referring to the use of the word "may" in the Pettis Application) is misguided and irrelevant to the enablement inquiry. The test for enablement is whether the ordinary skilled artisan (not the inventor) can use the guidance in the specification to practice the invention without undue experimentation -- the Applicant's alleged "uncertainty" does not factor into this equation. The evidence in this record shows the successful use of the claimed method for a good number of drugs (the Pettis Application itself; the first Pettis Declaration dated January 6, 2006), and that it could be practiced without undue experimentation (Kasting Decl., ¶¶ 13-16).

2. THE CLAIMS ARE NOT ANTICIPATED

Claims 29, 2, 3, 5-7, and 10-13 are rejected under 35 U.S.C. § 102(b) as anticipated by Gross *et al.* (U.S. Patent No. 5,848,991); Ganderton (U.S. Patent No. 3,814,097); or Autret (Autret et al., 1991, Therapie 46:5-8). The Examiner contends that Gross or Ganderton inherently anticipate the claims, and that Autret expressly anticipates the claims. For reasons detailed below, these rejections are erroneous and should be withdrawn.

2.1 The Claims Are Not Inherently Anticipated By Gross or Ganderton

The Examiner erroneously interprets Gross and Ganderton as disclosing the delivery of drugs to the intradermal compartment of a human subject's skin using a hollow needle having the features set forth in step (a) of claim 29, and incorrectly concludes that the resulting "plasma profile" (*sic*, pharmacokinetic profile) would be "inherently similar to, but higher as compared to subcutaneous injection." Taking the next leap of faith, the Examiner then asserts that one would somehow arrive at the right combination of needle length and pressure to inherently achieve the higher C_{max} and AUC recited in the claims (Office Action, p. 4). There is absolutely no proof in this record that practicing Gross or Ganderton would inherently achieve the PK profile required by the claims. In maintaining this inherency rejection, the Examiner notes that the Applicant has not shown any data to support the proposition that intradermal delivery would *not* inherently result in a higher C_{max} and AUC (Office Action, p. 6). The Applicants take this opportunity to supply the evidence requested by the Examiner and submit the Second Declaration of Dr. Pettis ("Second Pettis Decl.").

2.1.1 Mere Injection of A Drug To the IntraDermal Space Does Not Inevitably Result In The Pharmacokinetic Profile Claimed

Pharmacokinetics (PK) describes the concentration-time history of a drug in the body, and is typically represented graphically by plotting the concentration of the drug in the circulation over time. At least three parameters are typically used to characterize the PK for

delivery of a drug -- T_{max}, C_{max}, and AUC. T_{max} is the time required for the drug to reach a maximum serum concentration; C_{max} is the maximum (or peak) serum concentration of the drug reached within a given dose and route of administration; whereas the area under the serum concentration curve ("AUC") is a measure of bioavailability. (*See* Amendment dated July 10, 2003 at p.7). The claims require elevation of two of these three PK parameters as compared to subcutaneous delivery -- namely, C_{max} (a higher maximum plasma concentration), and AUC (a higher bioavailability); *e.g.*, claim 29.

The Second Pettis Declaration shows that mere injection of a drug to the intradermal compartment does *not inevitably* result in a higher C_{max} and AUC. Unless parameters are controlled -- as required by the claims -- delivery of substances to the intradermal space can result in an increased C_{max} and a decreased T_{max}, but nearly identical AUCs as compared to subcutaneous delivery (*see* Second Pettis Decl., ¶¶ 6-9); and in other cases, no difference between the C_{max} and AUC parameters is observed (Second Pettis Decl., ¶¶ 6, 7, 10 and 11).

While there is absolutely no evidence that practicing Gross or Ganderton would inherently result in the claimed PK profile, assuming *arguendo* these prior art methods were used to inject a drug into the intradermal space, the claimed PK profile would *not inevitably* result; *see*, *e.g.*, the Second Pettis Declaration.

In order for a prior art reference to inherently anticipate the claimed invention, the method disclosed must *inevitably* result in the claimed invention, *i.e.*, the claimed PK profile must be achieved *each time and every time* the methods of Gross and Ganderton are practiced. *In re Oelrich*, 666 F.2d 578, 212 U.S.P.Q. 323 (C.C.P.A. 1981); *Continental Can Co. USA Inc. v. Monsanto Co.*, 948 F.2d 1264, 1269, 20 U.S.P.Q.2d 1746 (Fed. Cir. 1991); *Glaxo Inc. v. Novopharm Ltd.*, 52 F.3d 1043, 34 U.S.P.Q. 2d 1565 (Fed. Cir. 1995). In other words, each and every time Gross or Ganderton are practiced, the methods *must* deliver the drug into the intradermal compartment so that systemic distribution having the clamed PK

profile is obtained. As shown by Dr. Pettis, injection into the intradermal space, without more, does not inevitably result in a PK profile having a higher C_{max} and AUC as required by the claims. (Second Pettis Decl., ¶¶ 6-11). Since neither Gross nor Ganderton would *inevitably* lead to the PK profile claimed, inherent anticipation cannot be found.

There are many more reasons for why Gross or Ganderton could not anticipate the claims; some of these are elaborated below.

2.1.2 The Systems Of Gross And Ganderton Will Not Deliver Drugs Selectively Into The Intradermal Compartment

Dr. Kasting, a scientist eminently qualified in the field of drug delivery, has compared the systems of Gross and Ganderton to the invention. Based on his evaluation, he has concluded that the Gross and Ganderton systems will not deliver drugs selectively to the intradermal space.

GROSS

Contrary to the Examiner's assumption, the claimed features for the structure and placement of the needle (step (a) of claim 29) are not disclosed in Gross. As elucidated by Dr. Kasting, Gross' specification does not provide guidance to the skilled practitioner on how to target the intradermal compartment to achieve the claimed PK profile. Gross does not teach using a needle having an exposed height of 0 to 1 mm so that the needle penetrates the intradermal compartment and the needle's outlet depth and exposed height of the outlet are located within the intradermal compartment, as required by the claimed invention. (Kasting Decl., ¶¶ 21, 22, and 23). Gross also fails to appreciate the requirement for applying pressure in amounts sufficient to control the rate of delivery. (Kasting Decl., ¶ 24). The Examiner has thus improperly attributed teachings from the Applicant's own disclosure into the prior art.

As confirmed by Dr. Kasting, Gross does not describe, measure or evaluate the systemic distribution or pharmacokinetic profile of any drug (Kasting Decl., ¶ 25).

Therefore, Gross does not expressly anticipate the pending claims. Moreover, since as previously explained, practicing Gross does not inevitably lead to the PK profile required by the claims, inherent anticipation cannot be found.

GANDERTON

The dressing disclosed in Ganderton does not have the correct configuration to deliver a drug to the intradermal compartment of human skin for systemic distribution and attainment of a desired pharmacokinetic profile, which is required by the claimed invention. Instead, Ganderton discloses a permeable pad studded with spikes (solid or hollow) used for *topical application*. The pad is applied to the skin so that the spikes disrupt the top layer (the stratum corneum). Drug applied on top of the permeable pad (with or without the application of pressure) diffuses through the permeable pad onto the disrupted skin.

As elaborated by Dr. Kasting, the spikes in the Ganderton dressing (even if 1000μm in length as suggested by the Examiner) would not penetrate the intradermal space even if pressure is applied. (Kasting Decl., ¶ 20). Dr. Kasting's analogy of the Ganderton dressing to a "bed of nails" illustrates the point well. The application of pressure to the device results in distributing the force over the area encompassed by the "bed of nails," so that the force is dissipated, not concentrated. As a result, the force applied to any one spike is correspondingly reduced below the level required to pierce skin. Thus, even if Ganderton's spikes were hollow needles made at the maximum length specified by Ganderton (*i.e.* 1000 μm), the application of pressure would not result in delivering the drug through the lumen of the needles into the intradermal compartment so that the claimed pharmacokinetic profile is achieved. Instead, the drug would diffuse through the permeable pad (including the holes pierced through the pad) resulting in topical application and diffusion through the epidermis. Thus, Ganderton could not achieve the claimed pharmacokinetic profile and does not anticipate the claimed method.

In sum, the dressing in Ganderton does not have the proper structure to penetrate the intradermal space nor does it provide adequate pressure to control the rate of delivery of the drug and thus omits elements of the claimed invention. Moreover, as confirmed by Dr. Kasting, Ganderton does not describe, measure or evaluate the systemic distribution or PK profile of any drug, and therefore does not expressly anticipate the pending claims (Kasting Decl., ¶ 20). Since practicing Ganderton does not inevitably lead to the PK profile required by the claims, inherent anticipation cannot be found.

2.1.3 Request For Examiner's Affidavit

There is no evidence on this record that practicing the methods of Gross or Ganderton would inherently result in delivering a drug having the PK profile claimed. Moreover, the evidence of record shows that the claimed PK profile would not inevitably result from practicing the prior art. In the event the Examiner disagrees, and to the extent that any rejection is based on facts within his personal knowledge, applicants request that the Examiner provide an affidavit pursuant to the provisions of 37 CFR § 1.104(d)(2).

2.2 Autret Does Not Anticipate The Claims

The Examiner's contention that Autret achieves a higher AUC than subcutaneous delivery is incorrect -- Autret expressly states, and the data in Autret shows (Autret, Fig. 1), that the AUC obtained from Autret's mesotherapy approach is *no different from that obtained by subcutaneous injection.* (*See* Kasting Decl., ¶ 19). Thus, the rejection must fail.

The Lebel needle used in Autret does not have the structural features required by claim 29 (*see* Kasting Decl., ¶ 18). Moreover, the PK profile disclosed by Autret is *not* the one claimed by the Applicants. As explained by Dr. Kasting, the PK profile disclosed by Autret (*see* Autret, Fig. 1) is virtually identical to the profile for subcutaneous delivery, and

does *not* exhibit *both* a higher C_{max} *and* higher AUC, as is required by the pending claims. On this point, Dr. Kasting notes that Autret's characterization of its own data supports the Applicant's position. (Kasting Decl., ¶ 19). Autret expressly states at p. 5, Summary, "[*n*]*either* mean plasmatic levels at each plasmatic dosage *nor* mean *areas under the curve* ... [*i.e.*, the standard measure of bioavailability]... were significantly different" when Autret's method was compared to the subcutaneous route of administration. (Emphasis supplied). As summarized by Autret, "[i]n this study ... [Autret's method and subcutaneous routes of administration] ... are *not different* with regard to plasma levels ...". (Emphasis supplied).

Here, where the claims positively recite the requirement for an increased AUC, and the prior art reference clearly and expressly states that it lacks that claim element (and actually shows the data) it is difficult to imagine how a case for anticipation could possibly be alleged.

It appears that the Examiner has applied an incorrect approach for determining AUC. Briefly, bioavailability is used to describe the fraction of an administered dose of a drug that reaches the systemic circulation. In order to determine the bioavailability of a drug, a pharmacokinetic study is done to plot the *plasma concentration of drug over time*. For an accurate measurement, blood is sampled frequently over a long enough time to observe virtually complete drug elimination. (Kasting Decl., ¶ 7). The bioavailability is measured by calculating the "area under curve" (AUC). In other words, the area of the PK profile spanning over the time points is calculated (represented in the figure below by the shaded region).

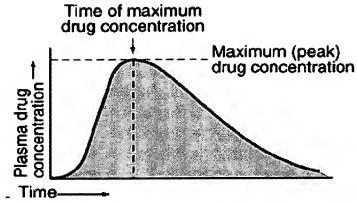

FIG. 298-1. Representative plasma concentration-time relationship after a single oral dose of a hypothetical drug. Area under the plasma concentration-time curve is indicated by shading. (Reproduced from Merck Manual of Diagnosis and Therapy, 1999, Clinical Pharmacology, reference CK of record).

In contrast to the art-accepted approach, the Examiner erroneously characterizes the AUC reported in Autret at a particular time point (1 hr) -- rather than the area under the curve (Office Action, p. 5, last paragraph). Clearly, AUC is *not* a measurement at one time point -- rather it is a measurement over a period of time. (*See* Kasting Decl., ¶ 7). Applying the proper mode of calculating AUCs to the PK profile of Autret, it is evident that the AUC of Autret's mesotherapy approach is *not* higher than the SC injection. This is confirmed by the authors of Autret and by Dr. Kasting (Kasting Decl., ¶ 19). The data do not support the Examiner's position, and the rejection based on Autret should be withdrawn.

3. THE CLAIMED INVENTION IS NOT OBVIOUS OVER GROSS IN VIEW OF PURI OR D'ANTONIO

Claims 29, 2, 3, 5-7, 10-13, and 16 are rejected under 35 U.S.C. §103(a) as obvious over Gross in view of Puri *et al.*, 2000, *Vaccine*, 18: 2600-12 ("Puri"), or U.S. Patent No. 6,056,716 to D'Antonio ("D'Antonio"). The Examiner contends that to the extent Gross does not inherently achieve the claimed pharmacokinetic profile -- this missing element is supplied

by Puri or D'Antonio. The obviousness rejection is based on the mistaken assertion that "Puri and D'Antonio disclose that intradermal injections give much greater C_{max} values than subcutaneous" (Office Action, p.5). The premise for this rejection is incorrect, and the rejection should be withdrawn.

Puri, which deals with vaccine delivery (not drugs) is concerned with the body's immune response to the vaccine -- in other words, how much antibody the body makes in response to vaccination -- not systemic distribution profiles, and certainly not C_{max} levels of the administered vaccine. To illustrate the point, at pp. 2609 - 2610 Puri describes an enhanced *immune response* -- as measured by a higher antibody response -- not an enhanced C_{max} and AUC of the vaccine substance as the Examiner contends!

D'Antonio relates to jet injection of vaccines and other substances -- not the intradermal delivery of drugs as claimed. Notably, at col. 29, line 3, cited by the Examiner, D'Antonio expressly states that the entire discussion (of the D'Antonio patent) focused on *intramuscular injection.* The remainder of that paragraph discusses the possibility of administering vaccines -- *not drugs* -- into the dermis, so that less antigen could be used to generate "an increasingly rapid and effective pick-up by the immune system" (D'Antonio, col. 29, ll. 23-26).

Unlike drugs, the efficacy and potency of vaccines are not evaluated using PK studies. By contrast, the efficacy of vaccines is typically evaluated by measuring their ability to confer a protective immunity in the host. Methods for assaying potency of immunogenic compositions such as vaccines include serologic testing such as measurement of antibody titers induced against the particular antigen. For example in Puri, an ELISA assay was developed to quantify antibody levels (not the injected vaccines) in the sera of immunized mice. Similarly, D'Antonio makes reference, not to a PK profile, but rather to a more rapid and effective pickup by the immune system.

The Examiner has improperly attributed parameters and properties of the drug delivery art to the vaccine art. Pharmacokinetic studies are meaningless in the vaccine art as practitioners in this field do not gauge the potency of the vaccine by its ability to be circulated systemically. In fact, as evidenced by the World Health Organization Guideline on Non-Clinical Evaluation of vaccine, pharmacokinetic studies, *e.g.*, determining serum or tissue concentrations of the vaccine are normally not needed and in fact shed no light on the efficacy of a vaccine.

Thus, neither Puri nor D'Antonio supply the pharmakinetic profile element missing from Gross; therefore, the combination does not render the claims obvious. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

4. MISCELLANEOUS

The Examiner's comments (Office Action, p. 6, first full paragraph) concerning the consistency of Applicant's arguments regarding the role of pressure in the claimed invention is not understood. Throughout this prosecution, the Applicants have maintained that the requirement of step (a) of claim 29 for inserting the needle, and the requirement of step (b) of claim 29 for pressure are critical for achieving the PK profile of the claims -- however, the absolute value of the applied pressure will vary depending upon the volume and nature of the formulation to be delivered. As explained in our previous response (January 6, 2005 at p. 7), in view of the guidance provided by the specification -- including the working examples -- the absolute value of the pressure used is not critical, in that it can be arrived at for a given formulation by a practitioner exercising ordinary skill. As verified by Dr. Kasting, the experimentation required is not "undue" (Kasting Decl., ¶¶ 13, 14, 15 and 16).

The Applicants hope that this discussion clarifies the matter raised by the Examiner.

5. CONCLUSION

The Applicant respectfully requests that the Examiner enter the amendments and consider the remarks made herein. Withdrawal of all rejections, and an allowance is earnestly sought. The Examiner is invited to call the undersigned attorney if a telephone call could help resolve any remaining items.

Respectfully submitted,

Date: October 7, 2005

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Express Mail No.: ED 181732845 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: RONALD J. PETTIS *et al.*	Confirmation No.: 7814
Serial No.: 09/606,909	Group Art Unit: 3763
Filed: June 29, 2000	Examiner: Michael J. Hayes
For: INTRADERMAL DELIVERY OF SUBSTANCES	Attorney Docket No.: 11219-008-999 (P-4901)

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.111

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Office Action mailed January 4, 2007 ("hereinafter "Office Action"), and pursuant to the provisions of Rule 111, please enter the amendments and consider the remarks below. Applicants submit herewith:

(i) a Third Declaration of Dr. Ronald J. Pettis under 37 C.F.R. § 1.132 with Exhibits A-E;

(ii) a Supplemental Information Disclosure Statement with List of References Cited by Applicant;

(iii) copies of References B03-B05 and C14;

(iv) a Petition for Extension of Time; and

(v) an Amendment Fee Transmittal Sheet.

The Commissioner is hereby authorized to charge any required fee(s) to Jones Day Deposit Account No. 503013.

Amendments to the Claims are reflected in the listing of claims which begin on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Canceled)

2. (Previously Presented): The method of Claim 29, wherein the needle is selected from the group consisting of microneedles, catheter needles, and injection needles.

3. (Previously Presented): The method of Claim 29, wherein a single needle is inserted.

4. (Previously Presented): The method of Claim 29, wherein multiple needles are inserted.

5. – 9. (Canceled)

10. (Previously Presented): The method of Claim 29, wherein the needle is about 300 μm to 2 mm long.

11. (Previously Presented): The method of Claim 29, wherein the needle is about 500 μm to 1 mm long.

12. (Previously Presented): The method of Claim 29, wherein the outlet is at a depth of about 250 μm to 2 mm when the needle is inserted.

13. (Previously Presented): The method of Claim 29, wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted.

14. (Canceled)

15. (Previously Presented): The method of Claim 29, wherein the outlet has an exposed height of about 0 to 300 μm.

16. (Previously Presented): The method of Claim 29, wherein the delivery rate or volume is controlled by spacing of multiple needles, needle diameter or number of needles.

17. (Withdrawn): A needle for intradermal delivery of a substance into skin comprising means for limiting penetration of the needle into the skin and an outlet positioned such that

when the needle is inserted into the skin to a depth determined by the penetration limiting means, leakage of the substance to the surface of the skin is substantially prevented.

18. (Withdrawn): The needle of Claim 17 wherein the outlet is at a depth of about 500 μm to 2 mm when the needle is inserted into the skin.

19. (Withdrawn): The needle of Claim 18 wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted into the skin.

20. (Withdrawn): The needle of Claim 17 which is about 300 μm to 2 mm long.

21. (Withdrawn): The needle of Claim 20 which is about 500 μm to 1 mm long.

22. (Withdrawn): The needle of Claim 17 which is contained in a device comprising a reservoir in fluid communication with the needle.

23. (Withdrawn): The needle of Claim 22 which is contained in a device further comprising pressure-generating means for delivering the substance through the needle.

24. (Withdrawn): The needle of Claim 23 wherein the pressure-generating means provides variable control of substance delivery rate.

25. – 28. (Canceled)

29. (Currently Amended): A method for administration of insulin ~~drug~~ to a human subject, comprising delivering the insulin ~~drug~~ through the lumen of a hollow needle into an intradermal compartment of the human subject's skin, which method comprises

(a) inserting the needle into the subject's skin so that the needle penetrates the intradermal compartment, and the needle's outlet depth and exposed height of the outlet are located within the intradermal compartment, wherein the outlet has an exposed height of about 0 to 1 mm; and

(b) delivering the insulin ~~drug~~ through the lumen of the needle with the application of pressure in an amount effective to control the rate of delivery of the insulin ~~drug~~,

so that the insulin ~~drug~~ is delivered through the lumen of the needle into the intradermal compartment and distributed systemically exhibiting ~~a pharmacokinetic profile similar to subcutaneous delivery of the drug, but with~~ a higher maximum plasma concentration and a higher bioavailability as compared to subcutaneous delivery.

30. – 31. (Canceled)

32. (Withdrawn): The method of claim 29, wherein the drug is used for the treatment of toxicity.

33. (Withdrawn): The method of claim 32, wherein the drug is an antitoxin.

34. (Withdrawn): The method of claim 29, wherein the drug is used to control pain.

35. (Withdrawn): The method of claim 34, wherein the drug is selected from a group consisting of an opioid, an analgesic, or an anesthetic.

36. (Withdrawn): The method of claim 29, wherein the drug is used to control thrombosis.

37. (Withdrawn): The method of claim 36, wherein the drug is selected from a group consisting of heparin, coumadin, or warfarin.

38. (Withdrawn): The method of claim 29, wherein the drug is used to control or eliminate infection.

39. (Withdrawn): The method of claim 38, wherein the drug is an antibiotic.

REMARKS

The outstanding Office Action is responsive to Applicants' amendment filed August 23, 2006. However, a Supplemental Amendment filed with the Office on December 22, 2006 containing certain amendments to the claims was, apparently, not entered or considered. Applicants believe that the amendments and remarks made in the Supplemental Amendment address certain issues in the outstanding Office Action. Since the Supplemental Amendment was not entered, the claims section of the present amendment reflects the changes made to the claims as of entry of Applicants' amendment filed August 23, 2006. Applicants request entry and consideration of the present amendment and remarks.

By the present amendment, claims 5-7 and 14 have been canceled without prejudice, and claim 29 has been amended. Accordingly, claims 2-4, 10-13, 15-24, 29, and 32-39 are pending in the application, with claims 17-24 and 32-39 standing withdrawn from consideration. Applicants have amended claim 29 for purposes of clarity to more particularly claim the intradermal delivery of insulin. Claim 29 as amended recites that the insulin is delivered through the lumen of the needle into the intradermal compartment and distributed systemically exhibiting a higher maximum plasma concentration and a higher bioavailability as compared to subcutaneous delivery. Applicants have amended the claims to expedite prosecution and allowance of the application. The amended claims are fully supported by the instant application, and no new matter has been added. Support for the amendment to claim 29 can be found, for example at page 4, lines 1-2 in combination with page 6, lines 2-6, and Figure 4.

The Claim Objection under 37 CFR 1.75(c) Has Been Rendered Moot

Claim 14 is objected to as being of improper dependent form for failing to further limit the subject matter of a previous claim.

In response, Applicants have canceled claim 14, thereby rendering the basis for the rejection moot. Applicants respectfully request withdrawal of the objection.

The Rejection of Claims 2-5, 10-16, and 29 under 35 U.S.C. § 112, First Paragraph as Failing to Comply with the Enablement Requirement Should Be Withdrawn

Claims 29, 2-5 and 10-16 are rejected under 35 U.S.C. § 112, first paragraph because the specification allegedly does not reasonably provide enablement for ID delivery of all drugs.

Without conceding to the propriety of the rejection, and solely to expedite prosecution of the present application, Applicants submit that the amended claims directed to intradermal delivery of insulin are fully enabled by the instant specification. As the Examiner has indicated in the Office Action mailed January 4, 2007, the specification is enabling for delivery of insulin to the intradermal compartment of the skin. The amendment has thereby rendered the asserted grounds for the rejection moot.

Applicants request reconsideration and withdrawal of the rejection for lack of enablement in view of the amended claims.

The Rejection of Claims 2-7, 10-16, and 29 under 35 U.S.C. § 112, Second Paragraph Has Been Rendered Moot

Claims 29, 2-7 and 10-16 are rejected under 35 U.S.C. § 112, second paragraph as indefinite. According to the Examiner, claim 29 recites that the pharmacokinetic profile between ID and SC injections is similar, but contends that the specification fails to clarify the similarities in ID and SC delivery methods, while at the same time showing a higher C_{max} and higher bioavailability.

Without conceding to the propriety of the rejection, and solely to expedite prosecution of the present application, Applicants have amended claim 29 to recite that the insulin is delivered through the lumen of the needle into the intradermal compartment and distributed systemically exhibiting a higher maximum plasma concentration and a higher bioavailability as compared to subcutaneous delivery. Accordingly, the amendments to the claims render the grounds for the rejection moot. Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, in view of the amended claims.

The Rejection of Claims 2-7, 10-16, and 29 under 35 U.S.C. § 103, Should Be Withdrawn

Claims 29, 2-7 and 10-16 are rejected under 35 U.S.C. §103(a) as obvious over Gross *et al.* (U.S. Patent No. 5,848,991, "Gross I") or Gross *et al.* (U.S. Patent No. 5,807,375,

"Gross II") in view of Prausnitz (U.S. Patent No. 6,611,707), Autret, Puri, D'Antonio *et al.* (U.S. Patent No. 6,056,716), Srivastava (U.S. Patent No. 6,007,821, "Prausnitz"), and The Merck Manual of Diagnosis and Therapy (17th ed.)(1999). The Examiner contends that to the extent that Gross is silent with respect to needle outlet exposed height and the pharmacokinetic profile of the ID delivered drug - - the missing elements are supplied by Prausnitz, Autret, Puri, D'Antonio, Srivastava and Merck.

The instant claims have been amended to recite a method of administrating insulin to the intradermal compartment comprising:

(a) administering via a needle to the intradermal compartment so that the needle's outlet depth and exposed height are within the intradermal compartment and wherein the outlet has an exposed height of about 0 to about 1 mm;

(b) delivering the insulin through the needle with the application of pressure to control the rate of delivery;

(c) so that when insulin is delivered it has a higher bioavailability and a higher plasma concentration as compared to subcutaneous administration.

The Examiner is relying on the combination of Gross and Prausnitz to provide the suggestion of claim elements (a) and (b); and is relying on Autret, Puri, D'Antonio, Srivastava and Merck Manual to provide the suggestion of claim element (c). However, the cited references are completely silent as to the needle configuration and needle placement as required by claim elements (a) and (b). The cited references are equally silent as to the improved pharmacokinetics resulting from intradermal administration as claimed. Finally, the claims have been amended to recite the administration of insulin, and none of the cited references describe improved pharmacokinetics of insulin when delivered intradermally. Thus, for the reasons discussed below, the cited references do not render obvious the claimed invention.

First, Gross does not describe the insertion of a needle so that *both* its outlet depth and exposed height of the outlet are located within the intradermal compartment of the subject's skin. Further, Gross does not describe a needle having an outlet with an exposed height of about 0 to 1 mm. These elements of the claims are not taught or suggested by Gross. Instead, Gross proposes methods and devices that *non-selectively* administer drugs below the epidermis, *i.e.*, to the interface between the epidermis and the dermis, or to the interior of the dermis or subcutaneously (*see* Gross at col. 3, *ll.* 38-45). In addition, not only is Gross silent

as to the claimed pharmacokinetic profile, practicing Gross does not inevitably result in the claimed profile.

Indeed, practice of the claimed method of intradermal delivery has a very different outcome as compared to delivery in accordance with Gross as demonstrated by the concurrently filed Third Declaration under § 1.132 of Dr. Ronald J. Pettis ("Declaration"). The Declaration demonstrates that delivery in accordance with the claimed configuration has a dramatic effect on the results obtained when compared to the results reported by Gross. Namely, administration in accordance with the claimed configuration dramatically affects the resulting pharmacodynamic profile, including both the rate and magnitude of the drop in blood glucose concentration as compared to Gross. *See*, Declaration.

As reported in Example 1 of Gross, when insulin was administered "intradermally" to rabbits, the rabbits' glucose levels dropped, and rose again when insulin administration ceased. However, rabbits receiving insulin in accordance with the claimed method demonstrated a much more precipitous drop in blood glucose concentrations. *See*, Declaration at ¶ 15 and Exhibits B, C, D, and E. In fact, as a result of intradermal administration of insulin in accordance via the claimed method at 100 IU/mL, the blood glucose concentrations of the test animals dropped to levels which could not be reversed by ceasing administration of insulin, nor by intervening through administration of glucose to the hypoglycemic test animals. This result indicates that there apparently was a high maximum plasma concentration and bioavailability of insulin when it was administered via the claimed method; hence the same hypoglycemic shock experienced by the test animals, as opposed to the result obtained by the methodology described in Gross, where the animals easily recovered once insulin administration ceased. Had Gross been practicing intradermal delivery as claimed in the instant application, the same result would have been observed; it was not. *See*, Declaration at ¶ 8.

One possible explanation for the differences in the observed pharmacodynamic response is that Gross fails to define the intradermal compartment, but merely describes delivery below the epidermal layer of the skin. Gross is devoid of any teaching relating to the configuration of the needle required to prevent leakage of the drug substance outside the intradermal space. It is the Applicants' disclosure, not Gross, which teaches the importance of not only the length of the needle, but the relative exposed height of the needle outlet (*e.g.*, the bevel) that could be used to successfully target the intradermal compartment. (*See*, specification at p. 5, *l.* 19-p. 6, *l.* 15). Unless the skin seals around the needle, the drug

substance will effuse out of the skin due to backpressure exerted by the skin itself, or the pressure built up from the accumulating fluid. The Applicants' specification sets forth principles and parameters relating to length of the needle and configuration of its outlet to prevent unwanted leakage. The Applicants' teachings also address mechanisms that can be used to provide adequate pressure so that the drug is efficiently and consistently delivered to the intradermal compartment of human skin where it is readily absorbed and systemically distributed. (For proper needle length and outlet configuration, *see*, instant specification at p. 5, *l.* 19-p. 6, *l.* 15; for proper pressure requirements to achieve intradermal delivery *see*, instant specification at p. 6, *l.* 16-p. 7, *l.* 4). In particular, the specification describes the use of microneedles that have *both* a length sufficient to penetrate the intradermal space *and* an *outlet depth within the penetration space* to allow the skin to seal around the needle to prevent effusion of the substance onto the surface of the skin due to backpressure *(see*, specification at p. 5, *l.* 33-p. 6, *l.* 3). Gross neither appreciates nor addresses the significance of these parameters for practicing the claimed method.

Contrary to the Examiner's position, these missing parameters from Gross are not provided by Prausnitz. Prausnitz describes a microneedle device for delivery across or into skin. However, for transdermal applications the "insertion depth" of the needle is preferably less than 100-150 μm, so that insertion does not even penetrate into the intradermal compartment (*see*, Prausnitz at col. 4, ll. 7-11). While Prausnitz does describe needles having overall lengths longer than 150 μm, Prausnitz specifically recites that the overall length of the needle is *not* equal to the inserted length. According to Prausnitz, the distal tip of the needle is *not* inserted into the skin and the actual length of the uninserted portion depends on the device design and configuration. (*see*, Prausnitz at col. 4, ll. 11-17). Thus, Prausnitz fails to describe a needle configured so that *both* its outlet depth and exposed height of the outlet are located within the intradermal compartment of the subject's skin.

The remaining references cited by the Examiner fail to describe or suggest the claimed needle configuration, and further fail to describe or suggest the claimed pharmacokinetic profile. In addition, given that the claims have been amended to cover administration of insulin, a number of the references cited by the Examiner are no longer relevant to the patentability of the pending claims.

For example, Autret is concerned with delivery of calcitonin, not insulin. Further, Autret concludes that the plasma levels of calcitonin resulting from intradermal and subcutaneous administration are *not different* (*see*, Autret, Summary at p. 5). Contrary to the

Examiner's assertion, Autret does *not* describe enhanced plasma levels resulting from intradermal delivery. As concluded by the authors of Autret, there is no difference in plasma levels of calcitonin when intradermal and subcutaneous delivery are compared.

Puri, D'Antonio and Srivastava are concerned with vaccine delivery - - not the delivery of insulin. The efficacy of a vaccine is measured by the ability of the body to mount an antibody response to the vaccine. Methods of assaying potency of immunogenic compositions such as vaccines include serologic testing such as measurement of antibody titers induced against the particular antigen. For example in Puri, an ELISA assay was developed to quantify antibody levels (not injected vaccine) in the sera of immunized mice. Similarly, D'Antonio makes reference, not to a PK profile, but rather to a more rapid and effective pickup by the immune system.

The Examiner has improperly attributed parameters and properties of the drug delivery art to the vaccine art. Pharmacokinetic studies are meaningless in the vaccine art as practitioners in this field do not gauge the potency of a vaccine by its ability to be circulated systemically. Thus, Puri, D'Antonio and Srivastava do not describe, nor do they suggest an enhanced bioavailability or plasma levels of insulin as required by the claims.

In sum, none of the references taken alone or in combination describe or suggest administration via a needle having the claimed configuration and placement in the intradermal compartment. The references taken alone or in combination are equally silent as to the improved pharmacokinetic profile – enhanced plasma levels and bioavailability of insulin when delivered intradermally. Accordingly, Applicants request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

Provisional Double Patenting Rejections Should Be Held In Abeyance

Claims 2-7, 10-16, and 29 are provisionally rejected on the non-statutory ground of obviousness-type double patenting over claims 8 and 10 of copending Application No. 10/868,482; claims 1, 2, 7, 8, and 50 of copending Application No. 10/867,908; claims 1-7, 9, 13, 16, 26, 28-30, 32, 35-41, 46-48, 50, 52-54, 57, 59, and 62-64 of copending Application No. 10/487,485; claim 25 of copending Application No. 11/004,780; claim 25 of copending Application No. 11/004,778; claims 1-3, 8, 10-16 of copending Application No. 10/841,992; claims 66 and 76 of copending Application No. 10/803,735; claims 22-26, 29-31, and 33 of copending Application No. 10/650,039; claim 33 of copending Appl. No. 10/249,973; claims 65, 71, 72, 75-77, and 82 of copending Application No. 09/893,746; claims 31, 32, 36, 37,

39, 49, 67, and 73 of copending Application No. 10/028,988; and claims 69, 72, 83-86, 88, 90, 100, and 103 of copending Application No. 10/028,989 in view of Gross '991 or Gross '375, and Prausnitz, Autret, Puri, D'Antonio, and Srivastava. As this is provisional obviousness-type double patenting rejection, Applicants respectfully request that the rejection be held in abeyance until such time as the Examiner indicates there is allowable subject matter, at which time the matter will be revisited in light of the allowable subject matter. Applicants respectfully submit that they will consider filing Terminal Disclaimers at which time allowable subject matter is identified. Based on the ability to file Terminal Disclaimers for each of the co-pending applications above and the statement filed above, Applicants respectfully request that the provisional double patenting rejections of claim 2-7, 10-16, and 29 be withdrawn and that claims 2-7, 10-16, and 29 be allowed.

CONCLUSION

Applicants respectfully request that the Examiner enter the amendments and consider the remarks made herein. Withdrawal of all rejections, and an allowance is earnestly sought. The Examiner is invited to call the undersigned attorney if a telephone call could help resolve any remaining items.

Respectfully submitted, /Eric A. Meade/ Eric A. Meade Reg No. 42,876

Date: June 18, 2007

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INTRADERMAL DELIVERY OF SUBSTANCES

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FIELD OF THE INVENTION

The present invention relates to methods and devices for administration of substances into the skin.

BACKGROUND OF THE INVENTION

Conventional needles have long been used to deliver drugs and other substances to humans and animals through the skin, and considerable effort has been made to achieve reproducible and efficacious delivery through the skin while reducing or eliminating the pain associated with conventional needles. Certain transdermal delivery systems eliminate needles entirely, and rely on chemical mediators or external driving forces such as iontophoretic currents or sonophoresis to breach the stratum corneum painlessly and deliver substances through the skin. However, such transdermal delivery systems are not sufficiently reproducible and give variable clinical results.

Mechanical breach of the stratum corneum is still believed to be the most reproducible method of administration of substances through the skin, and it provides the greatest degree of control and reliability. Intramuscular (IM) and subcutaneous (SC) injections are the most commonly used routes of administration. The dermis lies beneath the stratum corneum and epidermis, beginning at a depth of about 60-120 μm below the skin surface in humans, and is approximately 1-2 mm thick. However, intradermal (ID) injection is rarely used due to the difficulty of correct needle placement in the intradermal space, the difficulty of maintaining placement of the needle in the intradermal space, and a lack of information and knowledge of the pharmacokinetic profiles for many drugs delivered ID. In addition, little is known about fluid absorption limits in dermal tissue and the effect of depot time on drug stability. However, ID administration of drugs and other substances may have several advantages. The intradermal space is close to the capillary bed to allow for absorption and systemic distribution of the substance but is above the peripheral nerve net which may reduce or eliminate injection pain. In addition, there are more suitable and accessible ID injection sites available for a patient as compared to currently recommended SC administration sites (essentially limited to the abdomen and thigh).

Recent advances in needle design have reduced the pain associated with injections. Smaller gauge and sharper needles reduce tissue damage and therefore decrease the amount of inflammatory mediators released. Of particular interest in this regard are microneedles, which are typically less than 0.2 mm in width and less than 2 mm in length. They are usually fabricated from silicon, plastic or metal and may be hollow for delivery or sampling of substances through a lumen (see, for example, US Patent No. 3,964,482; US Patent No. 5,250,023; US Patent No. 5,876,582; US Patent No. 5,591,139; US Patent No. 5,801,057; US Patent No. 5,928,207; WO 96/17648) or the needles may be solid (see, for example, US Patent No. 5,879,326; WO 96/37256). By selecting an appropriate needle length, the depth of penetration of the microneedle can be controlled to avoid the peripheral nerve net of the skin and reduce or eliminate the sensation of pain. The extremely small diameter of the microneedle and its sharpness also contribute to reduced sensation during the injection. Microneedles are known to mechanically porate the stratum corneum and enhance skin permeability (US Patent No. 5,003,987). However, the present inventors have found that, in the case of microneedles, breaching the stratum corneum alone is not sufficient for clinically efficacious intradermal delivery of substances. That is, other factors affect the ability to deliver substances intradermally via small gauge needles in a manner which produces a clinically useful response to the substance.

US Patent No. 5,848,991 describes devices for the controlled delivery of drugs to a limited depth in the skin corresponding to about 0.3-3.0 mm and suggests that such devices are useful for delivery of a variety of drugs, including hormones. US Patent No. 5,957,895 also describes a device for the controlled delivery of drugs wherein the needle may penetrate the skin to a depth of 3 mm or less. The fluid in the pressurized reservoir of the device is gradually discharged under gas pressure through the needle over a predetermined interval, e.g., a solution of insulin delivered over 24 hrs. Neither of these patents indicates that delivery using the devices produces a clinically useful response. Kaushik, et al. have described delivery of insulin into the skin of diabetic rats via microneedles with a detectable reduction in blood glucose levels. These authors do not disclose the depth of penetration of the microneedles nor do they report any results suggesting a clinically useful glucose response using this method of administration. Further, there is no evidence of accurate or reproducible volume of delivery using such a device. WO 99/64580 suggests that substances may be delivered into skin via microneedles at clinically relevant rates. However, it fails to appreciate that clinical efficacy is dependent upon both accurate, quantitative, and reproducible delivery of a volume or mass of drug substance and the pharmacokinetic uptake and distribution of that substance from the dermal tissue.

SUMMARY OF THE INVENTION

The present invention improves the clinical utility of ID delivery of drugs and other substances to humans or animals. The methods employ small gauge needles, especially microneedles, placed in

the intradermal space to deliver the substance to the intradermal space as a bolus or by infusion. It has been discovered that the placement of the needle outlet within the skin is critical for efficacious delivery of active substances via small gauge needles to prevent leakage of the substance out of the skin and to improve absorption within the intradermal space. ID infusion is a preferred method for delivery according to the invention because lower delivery pressures are required. This also reduces the amount of substance lost to the skin surface due to internal pressure which increases as fluid accumulates within the skin prior to absorption. That is, infusion minimizes effusion of the substance out of the tissue. Infusion also tends to reduce painful swelling and tissue distension and to reduce internal pressure as compared to the corresponding bolus dose. The pharmacokinetics of hormone drugs delivered according to the methods of the invention have been found to be very similar to the pharmacokinetics of conventional SC delivery of the drug, indicating that ID administration according to the methods of the invention is likely to produce a similar clinical result (i.e., similar efficacy) with the advantage of reduction or elimination of pain for the patient. Delivery devices which place the needle outlet at an appropriate depth in the intradermal space and control the volume and rate of fluid delivery provide accurate delivery of the substance to the desired location without leakage.

DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the results of Example 1 for plasma insulin levels during SC and ID infusion of insulin.

Fig. 2 illustrates the results of Example 1 for blood glucose levels during SC and ID infusion of insulin.

Fig. 3 illustrates the results of Example 1 for plasma PTH levels during SC and ID infusion of PTH.

Fig. 4 illustrates the results of Example 2 for plasma insulin levels during SC and ID infusion of insulin at 2 U/hr.

Fig. 5 illustrates the results of Example 2 for plasma glucose levels during SC and ID infusion of insulin at 2 U/hr.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides delivery of a drug or other substance to a human or animal subject via a device which penetrates the skin to the depth of the intradermal space. The drug or substance is administered into the intradermal space through one or more hollow needles of the device. Substances infused according to the methods of the invention have been found to exhibit pharmacokinetics similar to that observed for the same substance administered by SC injection, but the

ID injection is essentially painless. The methods are particularly applicable to hormone therapy, including insulin and parathyroid hormone (PTH) administration.

The injection device used for ID administration according to the invention is not critical as long as it penetrates the skin of a subject to a depth sufficient to penetrate the intradermal space without passing through it. In most cases, the device will penetrate the skin to a depth of about 0.5-3 mm, preferably about 1-2 mm. The devices may comprise conventional injection needles, catheters or microneedles of all known types, employed singly or in multiple needle arrays. The terms "needle" and "needles" as used herein are intended to encompass all such needle-like structures. The needles are preferably of small gauge such as microneedles (i.e., smaller than about 25 gauge; typically about 27-35 gauge). The depth of needle penetration may be controlled manually by the practitioner, with or without the assistance of indicator means to indicate when the desired depth is reached. Preferably, however, the device has structural means for limiting skin penetration to the depth of the intradermal space. Such structural means may include limiting the length of the needle or catheter available for penetration so that it is no longer than the depth of the intradermal space. This is most typically accomplished by means of a widened area or "hub" associated with the shaft of the needle, or for needle arrays may take the form of a backing structure or platform to which the needles are attached (see, for example, US Patent 5,879,326; WO 96/37155; WO 96/37256). Microneedles are particularly well suited for this purpose, as the length of the microneedle is easily varied during the fabrication process and microneedles are routinely produced in less than 1 mm lengths. Microneedles are also very sharp and of very small gauge (typically about 33 gauge or less) to further reduce pain and other sensation during the injection or infusion. They may be used in the invention as individual single-lumen microneedles or multiple microneedles may be assembled or fabricated in linear arrays or two-dimensional arrays to increase the rate of delivery or the amount of substance delivered in a given period of time. Microneedles may be incorporated into a variety of devices such as holders and housings which may also serve to limit the depth of penetration or into catheter sets. The devices of the invention may also incorporate reservoirs to contain the substance prior to delivery or pumps or other means for delivering the drug or other substance under pressure. Alternatively, the device housing the microneedles may be linked externally to such additional components.

It has been found that certain features of the intradermal administration protocol are essential for clinically useful pharmacokinetics and dose accuracy. First, it has been found that placement of the needle outlet within the skin significantly affects these parameters. The outlet of a smaller gauge needles with a bevel has a relatively large exposed height (the vertical "rise" of the outlet). Although the needle tip may be placed at the desired depth within the intradermal space, the large exposed height of the needle outlet allows the substance being delivered to be deposited at a much shallower depth nearer the skin surface. As a result, the substance tends to effuse out of the skin due to backpressure exerted by the skin itself and to pressure built up from accumulating fluid from the

injection or infusion. For example, 200 μm microneedles are often cited as suitable means for delivery of substances through the skin. We have found, however, that even if the needle outlet is at the tip of such a microneedle (without any bevel) the substance is deposited at too shallow a depth to allow the skin to seal around the needle and the substance readily effuses onto the surface of the skin. Shorter microneedles such as these serve only to permeabilize the skin and do not give sufficient dose control for clinical utility. In contrast, microneedles according to the invention have a length sufficient to penetrate the intradermal space (the "penetration depth") and an outlet at a depth within the intradermal space (the "outlet depth") which allows the skin to seal around the needle against the backpressure which tends to force the delivered substance toward the skin surface. In general, the needle is no more than about 2 mm long, preferably about 300 μm to 2 mm long, most preferably about 500 μm to 1 mm long. The needle outlet is typically at a depth of about 250 μm to 2 mm when the needle is inserted in the skin, preferably at a depth of about 750 μm to 1.5 mm, and most preferably at a depth of about 1 mm. The exposed height of the needle outlet and the depth of the outlet within the intradermal space influence the extent of sealing by the skin around the needle. That is, at a greater depth a needle outlet with a greater exposed height will still seal efficiently whereas an outlet with the same exposed height will not seal efficiently when placed at a shallower depth within the intradermal space. Typically, the exposed height of the needle outlet will be from 0 to about 1 mm, preferably from 0 to about 300 μm. A needle outlet with an exposed height of 0 has no bevel and is at the tip of the needle. In this case, the depth of the outlet is the same as the depth of penetration of the needle. A needle outlet which is either formed by a bevel or by an opening through the side of the needle has a measurable exposed height.

Second, it has been found that the pressure of injection or infusion must be carefully controlled due to the high backpressure exerted during ID administration. Gas-pressure driven devices as are known in the prior art are prone to deviations in delivery rate. It is therefore preferable to deliver the substance by placing a constant pressure directly on the liquid interface, as this provides a more constant delivery rate which is essential to optimize absorption and to obtain the desired pharmacokinetics. Delivery rate and volume are also desirably controlled to prevent the formation of weals at the site of delivery and to prevent backpressure from pushing the needle out of the skin. The appropriate delivery rates and volumes to obtain these effects for a selected substance may be determined experimentally using only ordinary skill. That is, in general the size of the weal increases with increasing rate of delivery for infusion and increases with increasing volume for bolus injection. However, the size and number of microneedles and how closely together they are placed can be adjusted to maintain a desired delivery rate or delivery volume without adverse effects on the skin or the stability of the needle in the skin. For example, increasing the spacing between the needles of a microneedle array device or using smaller diameter needles reduces the pressure build-up from unabsorbed fluid in the skin. Such pressure causes weals and pushes the needle out of the skin. Small

diameter and increased spacing between multiple needles also allows more rapid absorption at increased rates of delivery or for larger volumes. In addition, we have found that ID infusion or injection often provides higher plasma levels of drug than conventional SC administration, particularly for drugs which are susceptible to *in vivo* degradation or clearance. This may, in some cases, allow for smaller doses of the substance to be administered through microneedles via the ID route, further reducing concerns about blistering and backpressure.

The administration methods contemplated by the invention include both bolus and infusion delivery of drugs and other substances to human or animal subjects. A bolus dose is a single dose delivered in a single volume unit over a relatively brief time period, typically less than about 5-10 min. Infusion administration comprises administering a fluid at a selected rate (which may be constant or variable) over a relatively more extended time period, typically greater than about 5-10 min. To deliver a substance according to the invention, the needle is placed in the intradermal space and the substance is delivered through the lumen of the needle into the intradermal space where it can act locally or be absorbed by the bloodstream and distributed systemically. The needle may be connected to a reservoir containing the substance to be delivered. Delivery from the reservoir into the intradermal space may occur either passively (without application of external pressure to the substance to be delivered) or actively (with the application of pressure). Examples of preferred pressure-generating means include pumps, syringes, elastomeric membranes, osmotic pressure or Belleville springs or washers. See, for example, US Patent No. 5,957,895; US Patent No. 5,250,023; WO 96/17648; WO 98/11937; WO 99/03521. If desired, the rate of delivery of the substance may be variably controlled by the pressure-generating means. As a result, the substance enters the intradermal space and is absorbed in an amount and at a rate sufficient to produce a clinically efficacious result. By "clinically efficacious result" is meant a clinically useful biological response resulting from administration of a substance. For example, prevention or treatment of a disease or condition is a clinically efficacious result, such as clinically adequate control of blood sugar levels (insulin), clinically adequate management of hormone deficiency (PTH, Growth Hormone), expression of protective immunity (vaccines), or clinically adequate treatment of toxicity (antitoxins). As a further example, a clinically efficacious result also includes control of pain (e.g., using triptans, opioids, analgesics, anesthetics, etc.), thrombosis (e.g., using heparin, coumadin, warfarin, etc.) and control or elimination of infection (e.g., using antibiotics).

EXAMPLE 1

ID infusion of insulin was demonstrated using a stainless steel 30 gauge needle bent at the tip at a 90° angle such that the available length for skin penetration was 1-2 mm. The needle outlet (the tip of the needle) was at a depth of 1.7-2.0 mm in the skin when the needle was inserted and the total exposed height of the needle outlet was 1.0-1.2 mm. The needle was constructed in a delivery device

similar to that described in US Patent No. 5,957,895, with infusion pressure on the insulin reservoir provided by a plastic Belleville spring and gravimetrically measured flow rates of 9 U/hr (90 μL/hr). The corresponding flow rates for SC control infusions were set using MiniMed 507 insulin infusion pumps and Disetronic SC catheter sets. Basal insulin secretion in swine was suppressed by infusion of octreotide acetate (Sandostatin®, Sandoz Pharmaceuticals, East Hanover, NJ), and hyperglycemia was induced by concommitant infusion of 10% glucose. After a two hour induction and baseline period insulin was infused for 2 hr., followed by a 3 hr. washout period. Plasma insulin levels were quantitated via a commercial radio-immunoassay (Coat-A-Count® insulin, Diagnostic Products Corporation, Los Angeles, CA), and blood glucose values were measured with a commercial monitor (Accu-chek Advantage®, Boehringer Mannheim Corp, Indianapolis, IN). Weight normalized plasma insulin levels and corresponding blood glucose values are shown in Fig. 1 and Fig. 2. Data indicate similar plasma insulin levels and onset periods for infusion via the ID route and via the conventional SC route. The decrease in blood glucose response is also similar between the two. Although 9 U/hr. is a higher administration rate than is typically used medically, these results also demonstrate the ability of dermal tissues to readily absorb and distribute medicaments which are infused via this pathway.

A similar experiment was conducted using human parathyroid hormone 1-34 (PTH). PTH was infused for a 4 hr. period, followed by a 2 hr. clearance. Flow rates were controlled by a Harvard syringe pump. Control SC infusion was through a standard 31 gauge needle inserted into the SC space lateral to the skin using a "pinch-up" technique. ID infusion was through the bent 30 gauge needle described above. A 0.64 mg/mL PTH solution was infused at a rate of 75 μL/hr. Weight normalized PTH plasma levels are shown in Fig. 3. This data demonstrates the efficacy of this route of administration for additional hormone drugs, and indicates that ID infusion may actually provide higher plasma levels for drugs that are susceptible to *in vivo* biological degradation or clearance.

EXAMPLE 2

ID insulin delivery was demonstrated in swine using a hollow silicon microneedle connected to a standard catheter. The catheter was attached to a MiniMed 507 insulin pump for control of fluid delivery.

A hollow, single-lumen microneedle (2 mm total length and 200 X 100 μm OD, corresponding to about 33 gauge) with an outlet 1.0 μm from the tip (100 μm exposed height) was fabricated using processes known in the art (US Patent No. 5,928,207) and mated to a microbore catheter commonly used for insulin infusion (Disetronic). The distal end of the microneedle was placed into the plastic catheter and cemented in place with epoxy resin to form a depth-limiting hub. The needle outlet was positioned approximately 1 mm beyond the epoxy hub, thus limiting penetration of the needle outlet into the skin to approximately 1 mm., which corresponds to the depth of the intradermal space in

swine. The patency of the fluid flow path was confirmed by visual observation, and no obstructions were observed at pressures generated by a standard 1 cc syringe. The catheter was connected to an external insulin infusion pump (MiniMed 507) via the integral Luer connection at the catheter outlet.

The pump was filled with Humalog™ (LisPro) insulin (Lilly) and the catheter and microneedle were primed with insulin according to the manufacturer's instructions. Sandostatin® solution was administered via IV infusion to an anesthetized swine to suppress basal pancreatic function and insulin secretion. After a suitable induction period and baseline sampling, the primed microneedle was inserted perpendicular to the skin surface in the flank of the animal up to the hub stop. Insulin infusion was begun at a rate of 2 U/hr and continued for 4.5 hr. Blood samples were periodically withdrawn and analyzed for serum insulin concentration and blood glucose values using the procedures of Example 1. Baseline insulin levels before infusion were at the background detection level of the assay, as shown in Fig. 4. After initiation of the infusion, serum insulin levels showed an increase which was commensurate with the programmed infusion rates. Blood glucose levels also showed a corresponding drop relative to negative controls (NC) and this drop was similar to the drop observed for conventional SC infusion (Fig. 5).

In this experiment, the microneedle was demonstrated to adequately breach the skin barrier and deliver a drug *in vivo* at pharmaceutically relevant rates. The ID infusion of insulin was demonstrated to be a pharmacokinetically acceptable administration route, and the pharmacodynamic response of blood glucose reduction was also demonstrated. This data indicates a strong probability of successful pharmacological results for ID administration of hormones and other drugs in humans according to the methods of the invention.

WHAT IS CLAIMED IS:

1. A method for delivering a substance into skin comprising delivering the substance into an intradermal space within the skin through a small gauge needle inserted into the intradermal space, wherein an outlet of the needle is inserted at a depth within the skin such that leakage of the substance to the surface of the skin is substantially prevented.

2. The method of Claim 1 wherein the needle is selected from the group consisting of microneedles, catheter needles, and injection needles.

3. The method of Claim 1 wherein a single needle is inserted.

4. The method of Claim 1 wherein multiple needles are inserted.

5. The method of Claim 1 wherein the substance is a liquid delivered by pressure directly on the liquid.

6. The method of Claim 1 wherein a hormone is delivered.

7. The method of Claim 6 wherein the hormone is selected from the group consisting of insulin and PTH.

8. The method of Claim 1 wherein the substance is infused.

9. The method of Claim 1 wherein the substance is injected as a bolus.

10. The method of Claim 1 wherein the needle is about 300 μm to 2 mm long.

11. The method of Claim 10 wherein the needle is about 500 μm to 1 mm long.

12. The method of Claim 1 wherein the outlet is at a depth of about 250 μm to 2 mm when the needle is inserted.

13. The method of Claim 12 wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted.

14. The method of Claim 12 wherein the outlet has an exposed height of about 0 to 1 mm.

15. The method of Claim 14 wherein the outlet has an exposed height of about 0 to 300 μm

16. The method of Claim 1 wherein delivery rate or volume delivered is controlled by spacing of multiple needles, needle diameter or number of needles.

17. A needle for intradermal delivery of a substance into skin comprising means for limiting penetration of the needle into the skin and an outlet positioned such that when the needle is inserted into the skin to a depth determined by the penetration limiting means, leakage of the substance to the surface of the skin is substantially prevented.

18. The needle of Claim 17 wherein the outlet is at a depth of about 500 μm to 2 mm when the needle is inserted into the skin.

19. The method of Claim 18 wherein the outlet is at a depth of about 750 μm to 1.5 mm when the needle is inserted into the skin.

20. The needle of Claim 17 which is about 300 μm to 2 mm long.

21. The needle of Claim 20 which is about 500 μm to 1 mm long.

22. The needle of Claim 17 which is contained in a device comprising a reservoir in fluid communication with the needle.

23. The needle of Claim 22 which is contained in a device further comprising pressure-generating means for delivering the substance through the needle.

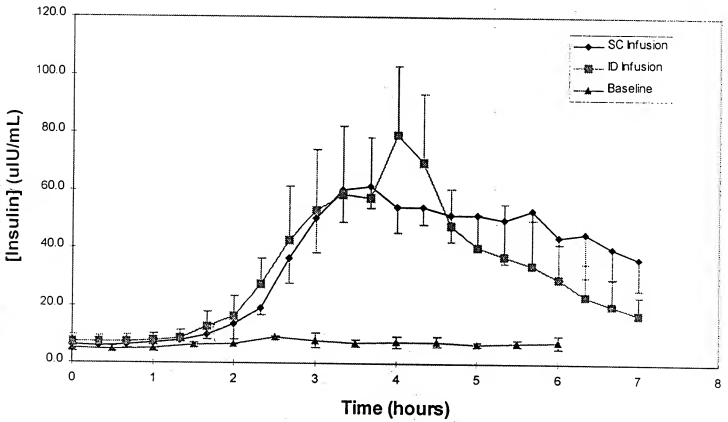
24. The needle of Claim 23 wherein the pressure-generating means provides variable control of substance delivery rate.

Add A'

Add B'

ABSTRACT

The present invention provides improved methods for ID delivery of drugs and other substances to humans or animals. The methods employ small gauge needles, especially microneedles, placed in the intradermal space to deliver the substance to the intradermal space as a bolus or by infusion. It has been discovered that the placement of the needle outlet within the skin and the exposed height of the needle outlet are critical for efficacious delivery of active substances via small gauge needles to prevent leakage of the substance out of the skin and to improve absorption within the intradermal space. The pharmacokinetics of hormone drugs delivered according to the methods of the invention have been found to be very similar to the pharmacokinetics of conventional SC delivery, indicating that ID administration according to the methods of the invention is likely to produce a similar clinical result (i.e., similar efficacy) with the advantage of reduction or elimination of pain for the patient. Delivery devices which place the needle outlet at an appropriate depth in the intradermal space and control the volume and rate of fluid delivery provide accurate delivery of the substance to the desired location without leakage.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100.0
80.0
60.0
40.0
20.0
0.0
[Insulin] (uIU/mL)
0
1
2
3
4
5
6
7
8
Time (hours)
SC Infusion
ID Infusion
Baseline

Fig. 1

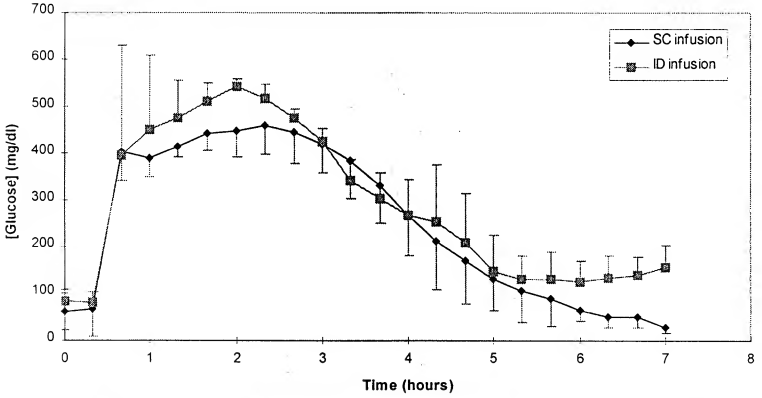
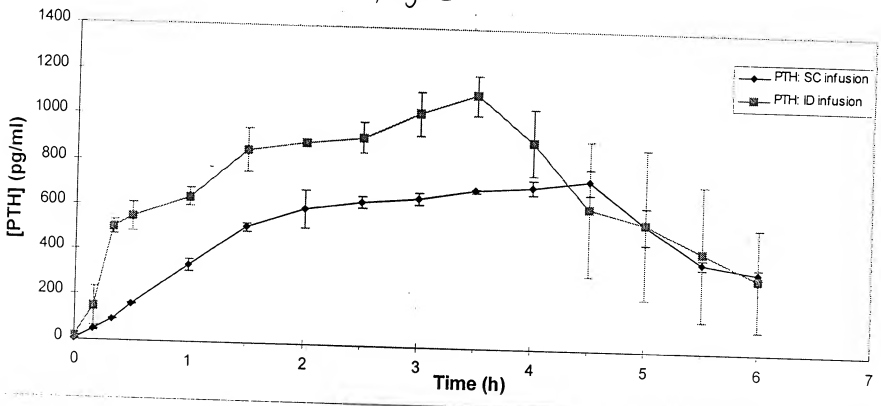

700
600
500
400
300
200
100
0
[Glucose] (mg/dl)
0
1
2
3
4
5
6
7
8
Time (hours)
SC infusion
ID infusion

Fig. 2

Fig.3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1200
1000
800
600
400
200
0
[PTH] (pg/ml)
0
1
2
3
4
5
6
7
Time (h)
PTH: SC infusion
PTH: ID infusion

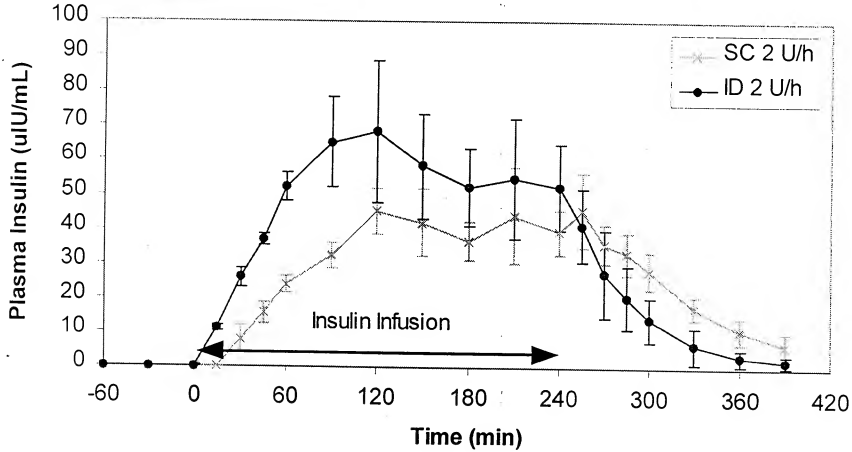

Fig. 4

Fig. 5